# Biennial and Annual Report on the Rare Diseases Research Activities at the National Institutes of Health

## FY 2004

Office of Rare Diseases

National Institutes of Health

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

www.nih.gov

JUL 27 2005

The Honorable Michael Enzi
Chairman, Committee on Health,
Education, Labor and Pensions
United States Senate
Washington, D.C. 20510

Dear Mr. Chairman:

I am pleased to submit to you the National Institutes of Health (NIH) Biennial Report and Plan on Rare Diseases Research Activities: FY 2004. This report also meets the requirement for our annual report to Congress found in section 404F of the Public Health Service Act, Public Law 107-280, the Rare Diseases Act of 2002.

This report presents the contributions and research advances of the NIH Institutes and Centers research programs and the Office of Rare Diseases (ORD). The basic, clinical, and research training programs contribute to the development and dissemination of information on the prevention, etiology, diagnosis, and treatment of rare diseases. Many advances presented in the report are the results of years of basic research sponsored by the NIH. Patients with rare diseases and their families continue to benefit from the treatment applications realized from the diverse nature of and emphasis placed on both basic and translational research by NIH.

Should you or your staff have any questions regarding the report please feel free to contact Dr. Stephen Groft, Director of ORD, at 301-402-4336.

Sincerely,

Elias A. Zerhouni, M.D.
Director

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

www.nih.gov

7 2005

The Honorable Edward M. Kennedy
Ranking Member, Committee on Health,
Education, Labor and Pensions
United States Senate
Washington, D.C. 20510

Dear Senator Kennedy:

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Bethesda, Maryland 20892

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JUL 7 2005

The Honorable Joe Barton
Chairman, Committee on Energy and Commerce
House of Representatives
Washington, D.C. 20515

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DEPARTMENT OF HEALTH & HUMAN SERVICES

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Bethesda, Maryland 20892

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JUL 7 2005

The Honorable John D. Dingell
Ranking Member, Committee on
Energy and Commerce
House of Representatives
Washington, D.C. 20515

Dear Mr. Dingell:

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# Biennial and Annual Report on the Rare Diseases Research Activities at the National Institutes of Health

## FY 2004

**Office of Rare Diseases**
**National Institutes of Health**
**Department of Health and Human Services**

**June 2005**

**Office of Rare Diseases**

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## Executive Summary[1]

The Rare Diseases Act of 2002, P.L. 107-280, instructs the Director of the Office of Rare Diseases, National Institutes of Health (NIH), to "biennially prepare a report that describes the research and education activities on rare diseases being conducted or supported through the national research institutes and centers, and that identifies particular projects or types of projects that should in the future be conducted or supported by the national research institutes and centers or other entities in the field of research on rare diseases." This first biennial report presents the contributions and research advances of the fiscal year (FY) 2004 NIH extramural and intramural research programs at the Institutes and Centers and of the Office of Rare Diseases (ORD) and other research offices in the Office of the Director of NIH as well as research plans for the near future.

Responses from the individual Institutes and Centers (ICs) provide an overview of ongoing rare diseases research activities, recent scientific advances in rare diseases research, new or planned rare diseases research initiatives, and rare disease-related activities such as scientific workshops and symposia, public and professional education and training, information dissemination, and other rare diseases research-related activities. Many advances presented are the direct result of years of rare diseases research sponsored by NIH in the past. Patients with rare diseases continue to benefit from the treatment applications realized by the emphasis NIH places on both basic and clinical intramural and extramural research programs. Many of the rare diseases activities conducted at the NIH Clinical Center have been reported by the Institutes/Centers (ICs) in their respective sections of the report. For example, the Bench-to-Bedside research program at the Clinical Center was reported by the Office of Rare Diseases. The Fogarty International Center (FIC) and the National Center on Minority Health and Health Disparities (NCMHD) did not have any rare diseases research activity to report.

This report uses the definition of rare diseases as set forth in the Orphan Drug Act and the Rare Diseases Act of 2002 as a disease or condition with a prevalence of fewer than 200,000 people in the United States. Prevalence refers to the number of individuals alive with the disease within a geographic parameter, i.e., the United States. There are more than 6,000 known rare diseases in the United States (see the rare diseases terms at http://ord.aspensys.com/asp/diseases/diseases.asp). Rare diseases are thought to affect approximately 25 million people in the United States. (Rare Diseases Act of 2002, Section 2, Findings.)

Activities undertaken in FY 2004 by the NIH ICs and the ORD included:

- Establishing a Trans-NIH Rare Diseases Research Working Group. For the coming year, the working group is developing plans for a conference on rare diseases biospecimen collection, storage, and delivery issues that impede research on rare diseases. Following the conference, the working group will develop an implementation plan. Other issues under review include the development of genetic tests by the ORD intramural programs and the NIH extramural research programs and an NIH-supported international conference on amyloidosis research.

[1] In the text of this report, common diseases may be included when particular subpopulations are rare or treatments are under development that are not expected to be financially recoverable.

! Expanding the ORD Rare Diseases Clinical Research Network by increasing the number of rare diseases clinical research consortia from 7 to 10. The network is described in detail later in this report. The network is cosponsored by the National Center for Research Resources (NCRR), National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institute of Child Health and Human Development (NICHD), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Heart, Lung, and Blood Institute (NHLBI), and National Institute of Neurological Disorders and Stroke (NINDS). The network consists of more than 70 sites and 30 patient advocacy groups that support research on almost 50 rare diseases with one central data technology and coordination center. At this time, 33 clinical protocols are under development and are approaching review by the Data Safety and Monitoring Board (DSMB) and implementation. In FY 2004, ORD added support for pilot studies and demonstration projects available to each consortium. Activities cosponsored in FY 2004 by the NIH ICs and ORD include the following new research activities:

- Publication of a Program Announcement by the NHLBI and the ORD for pilot studies, demonstration projects, and/or exploratory research studies in rare diseases;
- Development of a Request for Applications by the National Human Genome Research Institute (NHGRI), ORD, and National Institute on Drug Abuse (NIDA) for research training grants in genomics and proteomics;
- Development of a Program Announcement by the NINDS and ORD to improve treatment outcomes for lysosomal storage disorders;
- Publication of an NIH-wide Program Announcement to support grants to provide support for the planning of Phase III clinical trials;
- Support by the ORD of the NIDDK Biliary Atresia Research Consortium, which contains nine pediatric liver disease centers;
- Support of the ORD/NHGRI rare diseases intramural research program that promotes fellowship training in the areas of clinical and biochemical genetics focusing on rare diseases; fosters protocol-based initiatives into rare diseases not currently investigated in the intramural program; assists in the investigation of select, unique disorders of unknown etiology; and provides overall research support for diagnostics including genetic testing and therapeutics of rare diseases;
- Support of Bench-to-Bedside Grants in the NIH Clinical Center. With matching support from the ICs, ORD increased the number of grants from 5 to 20 grants annually;
- Cosponsorship by the ORD with NIH ICs of 87 scientific conferences in FY 2004 and, to date in FY 2005, 58 scientific conferences, the foci of which are listed later in this report. The ORD scientific conferences program continues to establish research priorities; develop program announcements; establish diagnostic and monitoring criteria; initiate the development of animal models; support development of patient and tissue registries, research protocols, and collaborative research arrangements; and disseminate workshop results through publications and other means;
- Expanding by ORD together with the NHGRI the reach of the Genetic and Rare Diseases Information Center by providing services in Spanish as well as English to

bring information about rare diseases to patients and their families, healthcare providers, researchers, and the public and implementing a user-friendly, Web-based application.

Since its inception in September 2001, the information center has responded with comprehensive, individualized responses to more than 10,000 inquiries about more than 3,000 different rare and genetic diseases.

## NATIONAL INSTITUTE ON AGING (NIA)

### Overview of Rare Diseases Research Activities

NIA maintains a vigorous portfolio of research in the area of rare diseases and conditions. Much of the work in this area focuses on the progeroid syndromes, including the Werner, Cockayne, Hutchinson-Gilford, and Rothmund-Thomson syndromes that have implications for age-related diseases. However, investigators are actively conducting research into the underlying molecular mechanisms and clinical manifestations of a diverse array of other diseases or conditions that fit into the classification of rare disorders. These include Bloom syndrome (BS), Fanconi anemia (FA), ATRX syndrome, Ehlers-Danlos syndrome, and premature ovarian failure.

*Cockayne Syndrome*

Cockayne syndrome (CS) is a rare human disease characterized by arrested postnatal growth, severe mental and growth retardation, microcephaly, progressive neurological and retinal degeneration, skeletal abnormalities, and a hypersensitivity to sunlight and results in premature aging and death. CS is an autosomal-recessive disorder that has been attributed to two genes—CSA and CSB. In recent experiments, NIA investigators have demonstrated that mutations in the CSB gene are the defect in DNA repair. The complex clinical phenotype of CS, however, suggests that DNA repair may not be the only defect. Studies have also demonstrated a significant transcription defect in CSB cells.

Recently, NIA scientists generated stable human cell lines with functional knockout of different regions of the CSB gene and demonstrated that the CSB protein plays a role in repair of oxidative DNA damage. Thus, this protein has several roles in DNA metabolism; it is involved in transcription, DNA repair, apoptosis, and chromatin assembly. Studies are now aimed at further structure/function analysis of CSB protein and aimed at further clarification of its function in these pathways.

The function of the CSB protein has also been investigated with microarray studies of gene expression. There are several genes that are underexpressed in mutated CS cells, and some of these confirm a substantial role for CSB protein in transcription and apoptosis.

In May 2004, the NIA, the NCI and the NIH ORD sponsored a 2-day workshop on Cockayne Syndrome and Related Disorders of DNA Repair and Transcription to discuss and address problems that impact patients with these diseases and to bring current research findings to clinicians and patients.

*Werner Syndrome*

Werner syndrome (WS) is a recessive genetic disease characterized by early onset of many characteristics of normal aging, such as wrinkling of the skin, graying of the hair, cataracts, diabetes, and osteoporosis. The symptoms of WS begin to appear around the age of puberty, and most patients die before age 50. Because of the acceleration of aging in WS, the study of this disease may shed light on the degenerative processes that occur in normal aging.

The WS gene has been identified (WRN), and defects are characterized by karyotypic abnormalities including inversions, translocations, and chromosome losses. Research is ongoing to elucidate the role of WRN protein, WRNp, in pathways of DNA metabolism and to define the protein interactions of WRN, which will help to elucidate cellular processes necessary to maintain genomic stability.

Cells from WS patients grow more slowly and undergo senescence at an earlier population doubling than age-matched normal cells, possibly because these cells appear to lose the telomeric ends of their chromosomes at an accelerated rate. In general, WS cells have a high level of genomic instability, with increased amounts of DNA deletions, insertions, and rearrangements. These effects could be the result of defects in DNA repair, replication, and/or recombination, although the actual biochemical defect remains unknown. Investigators have made purified WRNp for use in a number of basic and complex biochemical assays and are pursuing several avenues to identify and characterize the biochemical defect in WS cells. Their observations of functional protein interactions suggest that WRN protein is involved in DNA repair processes, in the pathways of base excision repair and of recombination. Investigators have found that the WRNp participates in processes at the telomere end and helps unwind the specific DNA substrates that are found there and interacts with proteins localized to those regions. Thus, an important function of the WRN protein is to maintain and stabilize the telomere regions and to help repair DNA lesions situated there. Investigators' ongoing and future studies will be directed toward elucidating the causes of the accelerated aging phenotype in WS, with hope that this knowledge can also be applied to our current understanding of both the aging of cells and organisms in general.

*Hutchinson-Gilford Progeria Syndrome*

Hutchinson-Gilford Progeria syndrome (HGPS) is a rare developmental disorder affecting most of the organ systems in a manner that mimics, to some extent, features of natural aging, but at a markedly accelerated rate. The individuals afflicted with this syndrome are clinically unaffected at birth, and the diagnosis is often not established until the second year of life. The diagnostic features include characteristic craniofacial disproportion, skin and hair abnormalities, loss of subcutaneous fatty tissue, and failure to thrive, resulting in short stature. These features give the patients a characteristic "old-like" appearance. Intellectual development is entirely normal. During the advancing years of the disease, the cardiovascular system becomes increasingly affected by atherosclerosis, and the patients die at an average age of 13 years from cardiovascular complications.

The NIA has continued to study HGPS because the identification of the culprit gene, designated as LMNA, has opened new avenues for research to explore the actual relationship between HGPS and normal aging. HGPS is a segmental progeria, and changes in lamin and nuclear structure might or might not happen during normal aging. In fact, while HGPS has been considered as a prototype of premature aging syndromes, the degree to which it truly recapitulates innate aging phenomena is still being studied.

The mode of inheritance, molecular basis, and pathogenic mechanisms of HGPS all remain elusive. Consequently, the NIA has an ongoing Program Announcement (PA-03-069) to encourage research on the biology of HGPS and other known laminopathies.

In April 2004, NIA cosponsored, together with the Progeria Research Foundation, a 2-day workshop to explore the potential for stem cell transplantation in HGS. While the participants generally agreed that it was too early to apply such an approach to patients, NIA is considering ways to encourage transplantation research in premature aging mouse models that have been produced within the past few years.

*Rothmund-Thomson and RAPADILINO Syndromes*

Rothmund-Thomson syndrome (RTS) is a rare disease associated with genome instability; predisposition to cancer, skin, and skeletal abnormalities; and some features of premature aging. The disease is caused by mutation in the RECQL4 gene—the same RecQ family that includes the WRN and BLM proteins defective in Werner and Bloom syndromes, respectively. An RTS-related disease, termed the RAPADILINO syndrome, with a lower predisposition to cancer, was found to be caused by mutations in the same *RECQL4* gene, however, the molecular mechanism of RTS and RAPADILINO syndromes is poorly understood.

The data suggest that RECQL4 may play a role in maintaining genomic stability and to understand the mechanism of this disease, NIA investigators have purified a human RECQL4 complex and identified its associated components; their work describes the first biochemical characterization of RECQL4 and its associated complex. The findings that RECQL4 is overexpressed and localized in the cytoplasm of multiple transformed cell lines are consistent with its role in maintaining genomic stability and suggest that it may be useful in cancer diagnosis. The discovery that RECQL4 is associated with UBR1 and UBR2, proteins that are ubiquitin ligases, is a hint to possible function; for example, the pathway in which these two proteins usually function is implicated in the regulation of chromosome stability and may be related to genomic instability of RTS patients.

*Fanconi Anemia*

FA is a recessively inherited disease characterized by congenital defects, bone marrow failure, and cancer susceptibility. Eight genes have been described that are mutated to cause FA; but many patients are not mutated in any of them and the mechanism underlying the FA pathway remains unclear, because most FA proteins lack recognizable structural features or any identifiable biochemical activity. Recent evidence suggests that FA proteins function in a DNA damage response pathway involving the proteins produced by the breast cancer susceptibility genes

BRCA1 and BRCA2. A key step in that pathway is a modification of an FA protein, FANCD2. Five other FA proteins (FANCA, -C, -E, -F, and -G) have been found to interact with each other to form a multiprotein nuclear complex, the FA core complex. Investigators have purified the FA protein core complex and found that it contains four new components in addition to the five known FA proteins. One new component of this complex, termed PHF9, is defective in a cell line derived from an FA patient and therefore represents a novel FA gene (FANCL). The work identifies the first FA gene that encodes a product with a catalytic activity, and the discovery of PHF9/FANCL could provide a potential target for new therapeutic modalities. More generally, the Fanconi pathway is involved in the DNA repair mechanisms that are often implicated as disrupted in cancer and possibly in aging.

NIA investigators are now studying whether three other components of the FA core complex are also novel FA genes. Current data show that, indeed, a specific subunit of the complex is defective in FA complementation group B patients (the gene is named FANCB). X-linked inheritance in this complementation group has important consequences for genetic counseling of FA-B families. Being present as a single active copy and essential for a functional FA pathway, FANCB is a potentially vulnerable component of the cellular machinery that maintains genomic integrity.

*Bloom Syndrome*

BS is a rare human genetic disease in which patients exhibit growth retardation, immunodeficiency, infertility, photosensitivity, and predisposition to cancer. The gene defective in BS has recently been cloned (named BLM). The recombinant BLM protein, BLMp, has been shown to possess a helicase activity *in vitro*, suggesting that BS could be caused by a defect in a DNA metabolic reaction, such as replication or repair. Interestingly, the BLM gene belongs to the helicase family, like the genes mutated in Werner and Rothmund-Thomson syndromes. All three diseases have some common features, such as genetic instability and predisposition to cancer. To understand the molecular mechanism of these human diseases, investigators will isolate the protein complexes containing each gene product.

One isolated complex contains five of the FA complementation group proteins. FA resembles BS in genomic instability and cancer predisposition, but most of its gene products have no known biochemical activity and the molecular pathogenesis of the disease is poorly understood. This work by NIA investigators provides the first biochemical characterization of a multiprotein FA complex and suggests a connection between the BLM and FA pathways of genomic maintenance. The findings that FA proteins are part of a DNA-unwinding complex imply that FA proteins may participate in DNA repair.

*ATRX Syndrome*

ATRX syndrome represents a combination of alpha-thalassemia, mental retardation, and multiple associated developmental abnormalities. The gene defective in ATRX has been localized to the X chromosome and cloned. Mutations in the same gene also cause several other forms of syndromal X-linked mental retardation, and it has been hypothesized that ATRX could function in an ATP-dependent chromatin-remodeling complex and participate in regulation of gene expression. Investigators have recently found that ATRX is in a complex with transcription cofactor Daxx, and

evidence supports that ATRX and Daxx are components of a novel ATP-dependent chromatin-remodeling complex. The defects in ATRX syndrome may result from inappropriate expression of genes controlled by this complex. Investigators have identified several sequence-specific transcription factors that co-purify with Daxx and are now studying if the genes regulated by these factors could be involved in ATRX syndrome.

*Ehlers-Danlos Syndrome / Hereditary Connective Tissue Disorders*

NIA investigators are examining the clinical and molecular effects of three well-known heritable disorders of connective tissue: Marfan syndrome, Ehlers-Danlos syndrome (EDS), and Stickler syndrome. Natural history data have been collected on subjects, including ophthalmologic, otolaryngologic, echocardiography, and rehabilitation medicine consultations. Investigators' studies have documented newly recognized gastrointestinal complications of these disorders and that chronic musculoskeletal pain is a significant complication of both EDS and Stickler syndrome. Echocardiography analysis of patients with EDS demonstrated a 30-percent incidence of aortic root dilation in this group of patients. Investigators have compared the Berlin and Gent nosologies for Marfan syndrome and examined the efficacy of screening for dural ectasia (increase in the connective tissue lining the spine) in the diagnosis of Marfan syndrome. Investigators have analyzed the prevalence of spinal and hip abnormalities in Stickler syndrome and their relationship to chronic pain. Their studies documented an increased risk of femoral head fracture in children with Stickler syndrome. Investigators have developed proposed diagnostic criteria for Stickler syndrome based on their clinical and molecular studies in this population. Researchers have also identified a previously undescribed connective tissue disorder with features resembling Marfan syndrome, Stickler syndrome, and EDS.

Investigators are also involved in several projects aimed at understanding the potential roles of alternative and complementary medical practices in the care of persons with genetic conditions. Many persons with Hereditary Connective Tissue Disorders (HDCT) suffer from chronic musculoskeletal pain. NIA investigators are designing studies aimed at understanding whether there are fundamental differences in the neurobiology of patients with HDCT that contribute to chronic pain. Interventions designed to ameliorate chronic pain in this population include mindfulness-based stress reduction in the Ehlers-Danlos population and "dry needling" of myofacial trigger points in patients with several different disorders of connective tissue. Investigators are also attempting to understand the role of connective tissue in the mechanism of acupuncture and to understand whether variations of connective tissue seen in the HDCT influence the efficacy of acupuncture.

*Premature Ovarian Failure*

Premature ovarian failure (POF) is a common condition, affecting one to three percent of all women, in which early menopause could result from inadequate formation or maintenance of the pool of ovarian follicles. NIA investigators previously found that the blepharophimosis-ptosis-epicanthus inversus syndrome (BPES), a syndrome associated with eyelid malformation as well as POF, is caused by mutations in FOXL2, a transcription factor. This provided an entry point for the study of ovarian development and POF.

Studies of *Foxl2* null mice point toward a new mechanism of POF. Mice lacking the gene recapitulate relevant features of human BPES: Males and females are small and show distinctive craniofacial morphology with upper eyelids absent. Furthermore, in mice as in humans, sterility is confined to females. More detailed analyses show that all major somatic cell lineages fail to develop around growing oocytes from the time of the first follicle formation. Thus, POF can arise from disrupted follicle development, with Foxl2 as a selective determinant of perinatal ovarian development.

*Foxl2* disruption in mice provides the first model directly relevant to POF in humans, along with a route to genes selectively involved in the determination of the critical follicle pool. Such genes should include candidates for mutation in other instances of POF, where affected genes have been difficult to identify. In the long run, they may provide targets for therapeutic intervention to reverse ovarian failure.

## NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM (NIAAA)

### Overview of Rare Diseases Research Activities

The National Institute on Alcohol Abuse and Alcoholism (NIAAA) conducts and supports research on the causes, consequences, prevention, and treatment of alcoholism and alcohol abuse. In addition to fetal alcohol spectrum disorders and various liver diseases, alcoholism is associated with and can be the cause of diseases targeting the lung, pancreas, heart, and other organs. The consequences of alcohol abuse and alcoholism are far reaching, so knowledge gained in related research programs will have broad application to other areas of human health and disease.

### Recent Scientific Advances in Rare Diseases Research

*Alcoholic Pancreatitis*

Long-term heavy alcohol consumption is associated with both acute and chronic pancreatitis. Progression of pancreatitis may lead to multiple co-morbidities including maldigestion, diabetes, and pancreatic cancer. NIAAA-funded researchers have made significant progress in understanding the underlying mechanisms by which alcohol intake leads to the development of pancreatitis.

*Role of Angiotensin II in Alcohol-Induced Pancreatic Fibrosis*

This study was undertaken to investigate the effects of angiotensin-converting enzyme inhibitor (captopril) and angiotensin II receptor antagonist (losartan) on alcohol-induced pancreatic fibrosis using an intragastric ethanol-feeding model of male rats. Dietary alcohol caused diffuse gland atrophy and interlobular and intralobular fibrosis with mild structural distortion in the pancreas, an effect that was blunted by captopril or losartan treatment. Alcohol also increased the number of alpha-smooth muscle actin-positive cells and transforming growth factor-beta (TGF-β) mRNA expression in the pancreas. These increases were blunted significantly by captopril or losartan treatment. These data suggest that angiotensin II contributes to the development of chronic alcohol-induced pancreatic fibrosis through its stimulation of TGF-β expression.

*Alcohol-Induced Alterations in the Pancreatic Exocrine Secretion*

Chronic alcohol ingestion appears to increase susceptibility of the pancreas to pancreatitis through modulating pancreatic exocrine secretion. This study was undertaken to determine the effect of chronic low- and-high dose alcohol consumption on the neurohormonal control of the exocrine pancreas in rats. Chronic alcohol ingestion was associated with a dose-related inhibition of basal pancreatic protein secretion, which was reversed upon alcohol withdrawal.

Low-dose alcohol feeding had no effect on bethanechol-stimulated pancreatic secretion but altered 2-deoxy-D-glucose (2-DG)-stimulated pancreatic secretion. In rats given chronic high doses of alcohol, meal- and bethanechol-stimulated protein secretion was significantly potentiated during early and late phases. The response to CCK appeared to be disinhibited, whereas the response to 2-DG was uniformly blunted. Upon withdrawal of low-dose alcohol, the response to 2-DG was

potentiated, whereas with the withdrawal of high-dose alcohol, the response to CCK was potentiated. Adaptation to chronic alcohol consumption differs depending on the alcohol dose. The most significant effects were seen after high-dose alcohol withdrawal, with apparent loss of central inhibitory regulation combined with exaggerated response at the acinar cell level. This combination of factors could increase susceptibility to acute alcoholic pancreatitis through a hyperstimulation mechanism.

*Fatty Acid Ethyl Esters Increase Extracellular Matrix Protein Levels in Pancreas*

Chronic alcoholic pancreatitis is characterized by fibrosis, which results from an excess of deposition of extracellular matrix (ECM) proteins in the pancreas; however, the mechanisms involved in ethanol-induced pancreas fibrosis are poorly understood. This study was designed to investigate the role of fatty acid ethyl esters (FAEE), nonoxidative ethanol metabolites, on ECM protein content in rat pancreas. The results demonstrated the FAEEs increased collagen, laminin, and fibronectin levels in pancreatic acini without affecting messenger RNA (mRNA) expression for these proteins. On the other hand, FAEEs reduced the activity of the serine protease, plasmin, and that of the urokinase-type plasminogen activator (uPA). Consistent with these results, the serine protease inhibitor aprotinin reproduced the effects of FAEEs and prevented the further increase in ECM proteins induced by FAEEs. *In vivo*, administration of FAEEs reduced plasmin and uPA activities and increased ECM protein levels in pancreas. These results suggest that FAEE-induced increases in ECM proteins in the pancreas are caused primarily by an inhibition in ECM degradation via serine proteases, including the plasminogen system.

*Alcohol-induced Hepatic Fibrosis*

Chronic heavy alcohol consumption is a major cause of liver cirrhosis, which ultimately results in death. Liver cirrhosis is a progression of fibrosis, which results from excessive deposition of ECM components, especially collagen, in the liver. Although various hepatic cells are involved in the development of fibrosis, hepatic stellate cells (HSCs) are the primary source of excessive ECM components. A major feature of fibrosis is the activation of HSCs, consisting of an early initiation phase followed by a perpetual phase. NIAAA-funded researchers have made the following progress in terms of understanding the mechanisms of HSC activation.

TGF-β *-mediated Reciprocal Expression of Type I Collagen and Matrix Metalloproteinase-13 Genes in Hepatic Stellate Cells*

TGF-β1 is a main cytokine involved in alcohol-induced liver fibrosis. It induces expression of the type I collagen genes in HSCs by a transcriptional mechanism, which is hydrogen peroxide dependent. In the present study, researchers investigated whether this cytokine is able to modulate the expression of collagen-degrading matrix metalloproteinase-13 (MMP-13) mRNA. The results showed that TGF-β1induces a rapid decline in steady-state levels of MMP-13 mRNA at the time that it induces the expression of α1 (I) collagen mRNA. In addition, TGF-β1-mediated effect is de novo protein synthesis dependent and requires the activity of p38MAPK, phosphatidylinositol 3-kinase, AKT, and p70 (S6k). These results suggest that TGF-β1 modulates the expression of type I collagen and MMP-13 mRNAs in HSCs in a reciprocal manner (increasing collagen but

decreasing MMP-13) that results in excess of collagen deposition in the liver—an important step in liver fibrosis.

*Role of Connective Tissue Growth Factor in Hepatic Stellate Cells Activation*

HSCs are the main source of increased collagen production that may result in hepatic fibrosis. Connective tissue growth factor (CCN2) is expressed during activation of HSCs and it promotes HSC proliferation, adhesion, and collagen production. This study was designed to investigate CCN2 signaling pathways in HSC. The results showed that CCN2 stimulated DNA synthesis and phosphorylation of FAK, Elk-1, and ERK1/2, the latter of which was blocked by heparin. The serum response element binding activity and luciferase reporter activity of the c-fos promoter, together with expression of c-fos, were enhanced by CCN2. CCN2-induced c-fos gene activation, expression, and cell proliferation were blocked by inhibiting ERK1/2 with PD98059. CCN2 promoter activity was enhanced by TGF-β1 or PDGF via a Smad7-dependent pathway. It was concluded that CCN2-stimulated HSC DNA synthesis is associated with transient induction of c-fos gene activation and expression as well as activation of the ERK1/2 signal pathway.

*Adipogenic Transcriptional Regulation of Hepatic Stellate Cells*

A major feature of fibrosis is the activation of hepatic stellate cells (HSCs) in which quiescent HSCs are transformed into collagen-producing activated HSCs, known as myofibroblast-like cells. The mechanisms of this transformation are not clear. In this study, researchers have demonstrated higher expression of adipogenic transcription factors such as C/EBP alpha, beta, and delta; PPARgamma; LXRalpha; SREBP-1c; and of adipocyte-specific genes in the quiescent HSCs, whereas increased expression of PPARbeta (transcription factor known to promote fatty acid oxidation) in activated HSCs was demonstrated. Furthermore, treatment of activated HSCs with the adipocyte-differentiation cocktail (MDI: isobutylmethylxanthine, dexamethasone, and insulin) or ectopic expression of PPARgamma or SREBP-1c in these cells induced a panel of adipogenic transcription factors, reduced PPARbeta, and caused the phenotypic reversal to quiescent HSCs. These results support the importance of adipogenic transcriptional regulation in HSC quiescence and provide a new framework for identifying novel molecular targets for the treatment of liver cirrhosis.

*Fetal Alcohol Syndrome and Other Alcohol-related Birth Defects*

Fetal alcohol spectrum disorders (FASD) describes a spectrum of prenatal alcohol effects resulting from drinking by the mother during pregnancy. The most serious disorder arising from prenatal alcohol exposure is fetal alcohol syndrome (FAS), a cluster of defects that includes mild craniofacial abnormalities, growth retardation, and central nervous system impairments manifested by deficits in executive function, memory and learning, and motor activity. FAS is considered the most common nonhereditary form of mental retardation. Alcohol-related neurodevelopmental disorder (ARND) is more variable in phenotype but can be equally debilitating. A variety of alcohol-related organ system birth defects have also been reported among children with FAS or ARND, including congenital heart defects, ocular abnormalities, and increased susceptibility to infections.

*Choline Supplementation and ARND*

Administering choline in the diet of postnatal rats that had been exposed to alcohol in the early neonatal period (equivalent to the third trimester of human pregnancy) was previously shown to enhance performance on a learning and memory task. Thus, choline supplementation appears to be a candidate for ameliorating deficits associated with FASD. Recent studies demonstrated that ethanol-induced motor deficits are not affected by choline supplementation. This highlights the fact that the effects of alcohol are multifaceted and that a single drug or supplement is not likely to be a panacea for the spectrum of FASD deficits. Drug discovery efforts may need to focus on the most debilitating behavioral deficits.

*Ethanol and Cell Death in the Developing Brain*

Ethanol has been shown to reduce the number of serotonin neurons during fetal development. Results from this study in cultured cells from rats strongly suggest that the neuronal loss is due to increased apoptosis, or programmed cell death. Apoptosis occurs in the course of the normal development of the nervous system. In addition to finding that ethanol increases apoptosis; the study also found that a serotonin-1A receptor agonist, which binds to and activates the receptor, protects the neurons by reducing apoptosis. This finding adds to the growing body of evidence that ethanol-induced cell death in a variety of developing neurons is mediated through apoptotic signaling pathways

*Promising Compounds to Prevent FAS*

The NIAAA continues to support research activities to find ways to prevent FASD. Scientists previously found that NAP and SAL, the active peptides from two brain proteins known to protect nerve cells against a variety of toxins, potently antagonize ethanol's teratogenic effects in a mouse model. Current studies revealed that the ethanol antagonist properties are not stereospecific; i.e., the synthetic D-stereoisomers were as effective as the L forms in preventing ethanol's effects. This was surprising, since most synthetic D-ligands and D-substrates are inactive. The D-stereoisomers are potentially attractive candidates for preventing FAS because of their relative resistance to proteases. Building on earlier findings that ethanol withdrawal causes neurotoxicity via the NMDA site on the glutamate neurotransmitter receptor during the human third trimester equivalent of development in rats, scientists treated ethanol-exposed animals during alcohol withdrawal with eliprodil, an NMDA receptor antagonist selective for the polyamine modulatory site. Subsequently, the animals showed improved performance on a learning test relative to alcohol-exposed animals that did not receive the drug. Thus, not only does this study support the hypothesis that ethanol withdrawal contributes to ethanol teratogenesis via NMDA receptor interaction, but it also suggests that eliprodil may be a promising compound for therapeutic development to prevent adverse ethanol effects during fetal development when it is administered during alcohol withdrawal.

**Significant Ongoing Rare Diseases Research Initiatives**

*Perinatal Alcohol, SIDS, and Stillbirth Initiative*

In collaboration with the NICHD, the NIAAA has launched an initiative to develop multi-disciplinary research projects in communities, both nationally and internationally, where prenatal maternal alcohol consumption is high to determine the relationship between prenatal alcohol exposure and other variables in the risk for sudden infant death syndrome (SIDS) and adverse pregnancy outcomes such as stillbirth and FAS. Pilot studies will be implemented in early 2005.

*Collaborative Initiative on FASD*

The NIAAA continues to support the CIFASD, a collaborative, multi-disciplinary, and cross-cultural research program aimed at developing effective interventions and treatment for FASD. The consortium coordinates basic, behavioral, and clinical investigations that utilize novel and cutting edge techniques. One of the first steps will be to definitively outline a diagnostic schema so that the full range of effects from exposure to moderate or large amounts of alcohol can be determined. The goal of the CIFASD is to bring together researchers from around the world who are conducting research on FASD or are interested in the global problem of FASD and who have the capabilities and resources to utilize international subject populations to further knowledge in this area. Advances in science often require the appropriate technological, social, and cultural climates to foster those advances. Studies that could not be conducted in any one site due to lack of subject numbers or given expertise will become possible through this collaborative initiative.

**Rare Disease-specific Conferences, Symposia, and Meetings**

**Mechanisms of Alcohol-associated Cancers**, **Bethesda, Maryland, October 6–7, 2004**. NIAAA organized this symposium in collaboration with ORD, ODS, NCI, NIDDK, and NIDA. The following topics were covered by 16 speakers: (1) general mechanisms of cancers; (2) epidemiology of alcohol-associated cancers; (3) alcohol and oral cancer; (4) cancers of upper aerodigestive tract and the large intestine; (5) acetaldehyde, microbes, and cancers of the digestive tract; (6) mechanisms of acetaldehyde-induced DNA damage; (7) alcohol and aldehyde dehydrogenase polymorphisms and cancer; (8) alcohol and hepatocellular carcinoma; (9) alcohol and pancreatic cancer; (10) role of alcohol in breast cancer; (11) marijuana and cancer; (12) nicotine and gastric cancer; (13) role of MAT and SAMe in alcohol-associated liver cancer; (14) alcohol, vitamin A, and cancer; (15) alcohol, iron-associated oxidative stress, and cancer; and (16) alcohol, folate, and cancer. The proceedings of the symposium will be submitted to a journal for publication.

*Mechanisms of Alcohol-induced Hepatic Fibrosis*

NIAAA has submitted a proposal to ORD for funding to support this symposium:

**Fetal Alcohol Syndrome and Other Alcohol-related Birth Defects, Heidelberg/Mannheim, Germany, September 28–October 2, 2004.** Dr. Kenneth Warren, Director of the NIAAA Office of Scientific Affairs, co-chaired this symposium as part of the 12th World Conference of the International Society for Biomedical Research on Alcoholism. Papers were presented on: Ophthalmological Involvement in the Fetal Alcohol Syndrome; Comparison of FAS in Moscow, Russia and San Diego, California; Neurobehavioral and Interventions for FASD in South Africa; and Brain Imaging Studies on Children with Fetal Alcohol Spectrum Disorders.

*Publications Related to ORD-sponsored Symposiums*

Purohit V, Russo D, Salin M, and Brown R (November 2003). Mechanisms of Alcoholic Pancreatitis: Introduction and Summary of the Symposium. *Pancreas* 27 (4): 281-285.Proceedings of the symposium (10 chapters), Mechanisms of Alcoholic Pancreatitis (November 2003). *Pancreas* 27 (4): 281-331.

Veech, Richard L., (March 2004). The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, Leukotrienes and Essential Fatty Acids 70(2004) 309-319.*

## NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES (NIAID)

### Overview of Rare Diseases Research Activities

The National Institute of Allergy and Infectious Diseases (NIAID) has a long history of research support for diseases that are classified as rare and has made considerable progress from basic discoveries in microbiology and immunology to the development of diagnostics, therapeutics, and preventive measures such as vaccines. Continued progress in this area of research will have considerable impact on the future of our Nation's public health and quality of life. NIAID supports research activities on rare diseases that are classified into four areas: infectious diseases, primary immunodeficiency diseases, autoimmune diseases, and other immune system-mediated conditions.

- Infectious diseases are caused by bacteria, viruses, fungi, protozoans, and other parasites.
- Primary immunodeficiency diseases are hereditary disorders caused by intrinsic defects in the cells of the immune system and are characterized by unusual susceptibility to infection.
- Autoimmune diseases result from a dysfunction of the immune system in which the body attacks its own organs, tissues, and cells.
- Other immune system-mediated diseases, such as asthma and allergic diseases, are characterized by immune responses that cause disease.

NIAID research on rare diseases seeks to delineate the immune mechanisms of disease pathogenesis and develop new and more effective strategies to diagnosis, treat, and prevent these diseases. Some of these diseases are considered Category A priority pathogens, which means that they pose the gravest threat should they be used as an agent of bioterror.

### Recent Scientific Advances in Rare Diseases Research

### Rare Infectious Diseases

*Amebiasis*

Amebiasis is the third leading cause of death worldwide, according to the World Health Organization (WHO). Infection with the pathogenic protozoan *Entamoeba histolytica* results in acute diarrheal illness and may also lead to a chronic condition that progresses to more severe symptoms including amebic colitis and liver abscess. The parasitic amoeba adheres to intestinal cells by binding to specific cell surface proteins, called lectins. Recently, NIAID-supported investigators hypothesized that a vaccine made from this lectin may interrupt parasite adherence, thus stopping the infection and preventing disease progression. Using a mouse model, these investigators found that vaccination with either native lectin or recombinant lectin was effective in preventing intestinal infection. If proven as effective in humans, this vaccine may ultimately reduce the transmission of amebiasis and prevent complications of the invasive disease in a significant number of people.

*Anthrax*

Anthrax is an acute infectious disease caused by the spore-forming, rod-shaped bacterium *Bacillus anthracis*, which is considered a Category A priority pathogen. By uncovering the molecular pathways that enable the bacterium to form spores, survive in people, and cause illness, NIAID hopes to identify new ways to diagnose, prevent, and treat anthrax. In FY 2004, NIAID-funded researchers determined the three-dimensional structure of the cell-binding component of anthrax toxin, called protective antigen (PA), binding tightly to a target cell surface protein called CMG-2. This finely detailed snapshot of the structure of the PA protein-CMG2 receptor complex offers insight into a crucial step in the pathway that allows anthrax toxin to enter human cells. This work provides important new leads for the development of novel antitoxins that could save lives late in the disease when large amounts of toxin are present and antibiotics are less effective.

In other research, NIAID-supported scientists discovered genes for toxins of the bacterium *B. anthracis* and an opportunistic pathogen, *Bacillus cereus*, which is known to cause food poisoning. The finding that the two species (*B. anthracis* and *B. cereus)* share genes including those that create the deadly anthrax toxin underscores the complexity of diagnosing anthrax since another species related to *B. cereus* causes an inhalation anthrax-like disease. This study also demonstrated that approaches such as rapid genome sequencing and analysis of clinical isolates can provide the genetic basis for an observed clinical phenotype.

*Botulism*

Botulism is caused by neurotoxins produced by *Clostridium botulinum*, a Category A priority pathogen. Research supported by NIAID seeks to detect neurotoxin DNA markers since current tests cannot distinguish the organism when it is releasing toxin from when it is not. The expression of recombinant botulinum neurotoxin heavy and light chain proteins has been hindered by their extreme lack of stability and solubility. NIAID-funded investigators have made advances in overcoming these obstacles and in expressing recombinant botulinum neurotoxin light chain fragments. The availability of recombinant fragments is important to the development of high-throughput assays that can be used to screen neurotoxin inhibitors. In addition, NIAID-supported investigators have described a subtype of serotype A neurotoxin, the “so called” A2 neurotoxin gene cluster in *C. botulinum*. Further characterization and understanding of serotype A toxins is essential to the development of countermeasures that will be broadly protective against the neurotoxin.

*Cholera*

The comma-shaped bacterium *Vibrio cholerae* causes severe diarrheal disease. Epidemics of cholera tend to follow blooms of chitin-producing zooplankton, and efforts to filter out zooplankton are protective against the disease. The basic unit of chitin is a monosaccharide, *N*-acetylglucosamine (GlcNAc) that acts as a chemoattractant to *V. cholerae.* GlcNAc induces expression of *V. cholerae* genes involved in its utilization by the bacterium. An NIAID-supported investigator and colleagues have described three genetic pathways encoding regulatory proteins in *V. cholerae* that are involved in control of chemotaxis towards GlcNAc, regulation of attachment, and utilization of chitin. The ubiquity of *Vibrio* species in the environment strongly suggests a

major role for this genus in the recycling of insoluble planktonic chitin and, therefore, in the global oceanic carbon cycle. Environmental changes that impact plankton growth such as increased nutrient levels and temperatures in coastal regions may be predictive of emerging cholera epidemics.

*Cryptococcosis*

Cryptococcosis, which is caused by the fungus *Cryptococcus neoformans*, is a life-threatening infection of the central nervous system (CNS) that commonly affects immunocompromised individuals. Cryptococcal meningoencephalitis (CM) develops as a result of inhaled *C. neoformans* traveling through the blood stream from the lung to the brain. Recently, NIAID-supported scientists, utilizing an animal model of CM infection, determined that the combination of a polyene [amphotericin B] with an azole [fluconazole] exhibited greater antifungal potency than amphotericin B alone, amphotericin B plus flucytosine, or fluconazole plus flucytosine. The treatment efficacy of the two-drug protocol demonstrated in this study is in sharp contrast to the clinical observation that amphotericin B and fluconazole are antagonistic when administered together. The data have contributed to the design of an international human clinical trial to address the need for more effective antifungal therapy for CM. Findings from an NIAID intramural study trace transfer of *C. neoformans* across the endothelial cells that form the blood brain barrier to invade the central nervous system.

*Dengue*

Dengue epidemics, which are caused by mosquito-borne transmission of the Dengue virus, increasingly pose public health problems in most tropical and subtropical countries. Secondary infections with the virus cause a hemorrhagic form of the disease. A safe and effective live dengue vaccine is still not available. Using an alternative vaccine strategy, NIAID intramural scientists developed a live, attenuated strain of the virus—which is now undergoing phase I clinical trials. In another study, NIAID-supported researchers recently identified the role of a gene that regulates egg production in response to blood meals in the mosquito, *Aedes aegypti,* that carries dengue and yellow fever. This discovery may reveal new strategies for reducing the ability of this mosquito species to reproduce.

*Epstein-Barr Virus*

Over 90 percent of persons in the United States are infected with the Epstein-Barr virus (EBV) and recover from the infection completely. Rarely, patients never recover and have a chronic, active infection with the virus that is ultimately fatal. NIAID intramural scientists have identified mutations in the gene encoding perforin that prevented its maturation in a patient with chronic active EBV infection. Perforin is important for destruction of certain virus-infected cells and the mutation was associated with impairment in this cell activity. The scientists identified that the mutation prevents maturation of the virus in a patient with chronic active EBV infection. Better understanding of the role of perforin and related proteins may lead to improved therapies for EBV-related infections and cancers.

*Escherichia Coli (Diarrheagenic)*

*Escherichia coli*, and other microbial infections, are increasingly difficult to treat because of the emergence of drug-resistant strains. Based on previous studies, NIAID-supported investigators developed an *in vitro* or test tube model of the human colon. The culture system, called the intestinal simulator, is a continuous-flow anaerobic culture that is inoculated with fecal samples from healthy volunteers. The resulting environment mimics the human gastrointestinal tract in terms of pH, oxygen tension, osmolarity, etc., and can be used to cultivate enteric pathogenic bacteria such as enteroaggregative *E. coli* (EAEC). The expression of bacterial genes, including factors that are necessary for bacterial virulence, can be measured and studied under these conditions. Interestingly, the nature of the normal bacterial flora contained in the intestinal simulator influenced the expression of EAEC genes. The generation of such a system is important because: (1) no animal models exist for many pathogens that cause diarrhea; (2) many pathogens are host species specific; and (3) this system mimics the human intestine.

*Ebola*

Ebola virus, a rare and deadly microbe that causes hemorrhagic fever, is characterized by high fever and massive internal bleeding. Ebola is considered a Category A priority pathogen. The first human trial of a vaccine designed to prevent Ebola infection was initiated in FY 2004 by the NIAID Vaccine Research Center (VRC). The candidate DNA vaccine is synthesized using modified, inactivated genes from Ebola virus and does not contain any infectious material. This gives the immune system information about viral structures so that it can mount a rapid defense should the real virus ever be encountered. In addition, the VRC is currently testing a fast-acting candidate Ebola vaccine. A single injection of this fast-acting, experimental Ebola vaccine successfully protected monkeys after only 1 month. In this study, VRC scientists immunized eight monkeys with a single-boost injection, consisting of attenuated adenovirus containing genes for important Ebola antigens. The monkeys were then delivered to the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), where they were injected with an Ebola virus strain obtained from a fatally infected person from the former Zaire in 1995. The single vaccine injection completely protected all eight animals against Ebola infection, even those animals receiving high doses of the virus. This finding suggests that it might be possible to quickly contain Ebola outbreaks with ring vaccination, which aims to contain an outbreak by inoculating all identified contacts of the first detected cases and contacts of contacts as well. Ultimately, VRC scientists envision a possible two-stage vaccination strategy called prime-boost: After "priming" with the DNA vaccine, the immune system response is "boosted," or augmented, by a second inoculation with the adenoviral component. The booster essentially sets the immune system on alert against future infection by the Ebola virus.

*Giardiasis*

*Giardia intestinalis* is a very common protozoan parasite that causes diarrhea. The disease is especially serious in developing countries, where people are frequently malnourished. Recently, scientists discovered that *Giardia*, which possess a nucleus, also possess small mitochondria-like organelles, which they named "mitosomes." These mitosomes were identified using molecular genomic techniques and were then located using immunohistochemistry and electron microscopy.

The presence of these organelles, which differ significantly from the mitochondria found in human cells, helps to explain why current drugs of choice are effective (e.g., metronidazole) and may form the basis for developing novel drugs and vaccines.

*Histoplasmosis Capsulatum*

The fungus *Histoplasma capsulatum* is a significant cause of potentially fatal infection in persons with weakened immune systems such as individuals with HIV/AIDS. Current therapies are often inadequate and cause liver failure or other problems that make it necessary to halt treatment. Now, NIAID-supported investigators have developed a specific antibody that protects mice from infection with *H. capsulatum*. This monoclonal antibody recognizes a protein on the yeast surface and directs the host's white blood cells to engulf the fungus. While *H. capsulatum* normally survives in the cells that engulfed it, these cells are killed more quickly because the antibody recognizes yeast epitopes that appear on its surface. This is the first demonstration of the use of an antibody to protect against *H. capsulatum* infection.

*Hepatitis E Virus*

The identification of antibodies to Hepatitis E virus (HEV) and the mapping of their corresponding neutralization epitopes enabled NIAID intramural scientists to develop an enzyme-linked immunosorbent assay (ELISA) that specifically detects antibodies that neutralize HEV. With this tool, scientists can now evaluate results of vaccine trials and improve vaccine formulations and delivery schedules. Availability of this test should decrease the number of monkeys needed for efficacy studies and may provide insight into the natural history and clinical course of HEV.

*Leishmaniasis*

Leishmaniasis is a parasitic disease that infects more than 12 million people on several continents, and another 400,000 people are at risk for infection. At this time, there is no effective vaccine against human cutaneous leishmaniasis. Based on results of previous studies, scientists believe that long-term immunity to *Leishmania* requires continued presence of the parasite; however, attempts to create attenuated (with reduced virulence) parasites that persist in the host and confer protection without reverting to a virulent state have shown limited success. Now, NIAID-supported investigators have designed a vaccine using parasites that lack a key *Leishmania* protein, lpg2, and administered it to mouse models that are normally susceptible to *Leishmania* infection. When these mice were later challenged with *Leishmania major* protozoa, they were protected from infection without suffering overt cutaneous lesions. Although more studies are necessary, this type of vaccine may prove to be useful in treating human disease. In addition, in an intramural study using massive cDNA sequencing, proteomics, and computational biology approaches, NIAID scientists isolated the most abundant secreted proteins from the salivary glands of the sand fly, *Lutzomyia longipalpis*, a *Leishmania* vector. Sixteen of these proteins appear to be unique to sand flies. Because of the relationship between the vertebrate host immune response to salivary proteins and protection from infection with the parasite, these proteins are promising markers for vector exposure and attractive targets for vaccine development to control *Leishmania chagasi* infection.

The protozoan parasite *Leishmania major* is transmitted through sandflies to humans, where it invades macrophages (cells that ingest and digest foreign substances) and may result in the debilitating disease known as visceral leishmaniasis. The *Leishmania* LACK antigen is a key target of immune response in susceptible mouse models of the disease and is being studied as a potential drug target to treat leishmaniasis. Drugs now used to treat leishmaniasis are very toxic and scientists are studying ways to improve management of the disease. In an NIAID-supported study, researchers found that, although they were able to target and select individual LACK genes in the organisms, the parasites remained attenuated, even in normally very susceptible mice. This encouraging finding supports the hypothesis that the LACK protein may be a useful target for therapeutic, if not immunologic, intervention against *leishmaniasis*.

NIAID intramural scientists also identified a sand fly receptor that is critical for the survival of *Leishmania major* in the midgut of the sand fly vector. This identification of an insect molecule central to survival and transmission of the protozoan parasite opens new avenues for studies of insect immunity, transmission-binding vaccines, and host-parasite co-evolution.

*Lyme Disease*

NIAID intramural scientists identified for the first time using direct molecular genetic investigation the individual genes required by the bacterium *Borrelia burgdorferi,* the causative agent of Lyme disease, at each stage of the natural mouse-tick infection cycle. NIAID scientists demonstrated an important developmental sequence of phenotypic changes accompanying transmission that previously was not recognized for this vector-borne bacterial pathogen. The conclusion is novel because adaptive responses of bacteria that do not undergo obvious morphological changes, such as *B. burgdorferi*, are generally believed to be of immediate rather than delayed utility. In another study, NIAID intramural scientists collaborated with several extramural scientists to show that the amount of antibody against C6, a protein fragment from *B. burgdorferi*, declines after successful antibiotic therapy of Lyme disease. These results suggest that a change in the anti-C6 antibody titer may serve as an indicator of therapy outcome for patients with Lyme disease. Further studies with more patients are needed to clarify how the test may be used in the clinical setting.

*Pertussis*

Pertussis (whooping cough) is a preventable illness in all age groups but is rarely considered or diagnosed in older children or adults. An NIAID-supported investigator and colleagues are studying the role of pertussis toxin in *Bordetella pertussis* infection leading to the disease pertussis. Pertussis toxin is considered to be an important factor produced by this bacterium that allows it to cause disease, but its role in the infection process has not been well studied. Using mouse infection studies, these investigators showed that the toxin inhibits immune responses, suggesting the possibility that *B. pertussis* infection in both children and adults may render them more susceptible to secondary infections and diseases, particularly respiratory diseases.

*Plague*

Plague, which is considered a Category A priority pathogen, is caused by the bacterium *Yersinia pestis* and is transmitted to people mainly through a flea bite. An experimental plague vaccine proved 100-percent effective when tested in a new mouse model for plague infection developed by NIAID scientists at Rocky Mountain Laboratories (RML). The scientists developed their model to mimic the natural transmission route of bubonic plague through the bites of infected fleas. The scientists will use the natural challenge model to test other plague vaccines in development. They also will try to learn how plague bacteria spread through a host after being transmitted by a flea, with hopes of developing new treatments to counteract the spread of plague in infected persons.

*Q Fever*

*Coxiella burnetii* is an obligate intracellular bacterium and the causative agent of the zoonosis Q fever. Recently, NIAID intramural scientists developed a model to study *C. burnetii* morphogenesis that uses a specific cell line that is synchronously infected with homogeneous small cell variants harvested from aged infected cell cultures. This experimental model to study *C. burnetii* development will dramatically aid future efforts to define the transcriptome and proteome of developmental forms. It will also allow a thorough assessment of the biological properties of cell forms in terms of infectivity, antibiotic sensitivity, and resistance to environmental insult.

*Severe Acute Respiratory Syndrome*

The severe morbidity and mortality associated with severe acute respiratory syndrome (SARS), and the concern that SARS coronovirus (SARS CoV) could reemerge, make it imperative that effective means to prevent and treat the disease are developed. To this end, NIAID scientists, grantees, and industry partners are hard at work to better understand SARS and the virus that causes it and to develop effective countermeasures against this disease. Animal models of disease are critical for the development of vaccines, immunotherapeutics, and antiviral drugs. NIAID scientists and their collaborators developed several animal models for SARS, including mouse, hamster, and non-human primate models. They also determined that the transfer of immune sera from infected mice and hamsters, which contained antibodies against SARS-CoV, protected uninfected animals from SARS infection. These observations suggest that vaccines that induce neutralizing antibodies against SARS-CoV and immunoprophylaxis or immunotherapy with anti-SARS antibodies are likely to be effective against the SARS-CoV. In other efforts to evaluate the potential of immunotherapy, NIAID-funded researchers have developed a human monoclonal antibody that reduces viral replication in mice and protects them against SARS-CoV challenge.

NIAID scientists collaborated with scientists at academic institutes and in industry to develop and evaluate the immunogenicity and efficacy of vaccines against SARS-CoV in the newly developed SARS animal models. Since it is not known what type of vaccine will be most effective against the SARS-CoV, several technologically distinct methods were initiated in parallel. NIAID researchers have developed three candidate SARS vaccines, a DNA vaccine and two live attenuated virus vaccines. Scientists in the NIAID's VRC have tested a DNA vaccine against SARS CoV and shown that it provides protection in an animal model. This DNA encodes part of the SARS virus

spike protein (which protrudes from the viral envelope and helps to bind the virus to its target cells) and protects mice from virus growth in the lungs by inducing neutralizing antibodies. A phase I clinical trial of this recombinant DNA vaccine is planned for December 2004.

The attenuated live virus vaccines were made by inserting the gene encoding the SARS-CoV spike protein into two existing vaccines, modified vaccinia Ankara (MVA), which was initially developed as a vaccine against smallpox and has an excellent safety record in humans, and a recombinant attenuated human parainfluenza virus 3 (BHPIV3), which is an experimental vaccine against HPIV3, a virus that can cause respiratory illness in children. The MVA and BHPIV3 vaccines act to transport the SARS gene into the body. The MVA/S vaccine was tested in mice and the BHPIV3/S vaccine in African green monkeys. Both experimental vaccines protected the immunized animals from infection with SARS-CoV.

Whether any of these experimental SARS vaccines will protect humans against the SARS-CoV is not yet known; similarly, it is not yet known if transfer of "passive immunity" through anti-SARS antibody administration can effectively treat or prevent human SARS. NIAID intramural and extramural researchers, in collaboration with researchers worldwide, are continuing to follow up on promising leads.

*Smallpox*

Smallpox is a disfiguring and potentially deadly infectious disease caused by the *Variola major* virus and is a Category A priority pathogen. Approximately 25 percent of the U.S. population cannot receive the current, licensed smallpox vaccination (Dryvax) because they are at increased risk for post-vaccine complications. MVA, a much weaker form of the same virus used to make Dryvax, was used briefly in Germany and Turkey in the 1970s as a primer before immunization with the Lister smallpox vaccine. NIAID scientists and their collaborators compared immunization with MVA and Dryvax in a monkey model. After two doses of MVA or one MVA dose followed by Dryvax, the immune response was equivalent or higher than that induced by Dryvax alone. These findings are important steps in the evaluation of MVA as a replacement vaccine or pre-vaccine for those with increased risk of severe side effects from Dryvax. In addition, NIAID intramural scientists found that MVA also protects mice with certain immune deficiencies, indicating that MVA may be a promising alternative to Dryvax in humans who are partially immunodeficient.

The A28L gene of vaccinia virus is conserved in all poxviruses and encodes a protein that is anchored to the surface of infectious intracellular mature virions (IMV) and consequently lies beneath the additional envelope of extracellular virions. In an NIAID-supported study, investigators found that A28-deficient virions did not induce cytopathic effects, express early genes, or initiate a productive infection. Although A28-deficient IMV bound to the surface of cells, their cores did not penetrate into the cytoplasm. Because repression of A28 inhibited cell-to-cell spread, all forms of vaccinia virus regardless of their outer coat must use a common A28-dependent mechanism of cell penetration. Furthermore, since A28 is conserved, all poxviruses are likely to penetrate cells in a similar way. The identification of a poxvirus entry protein will facilitate the identification of additional components of the fusion complex, which will ultimately

lead to an understanding of the entry process. In addition, antibodies and drugs that target A28 may be useful for vaccines and therapeutics.

*Streptococcal Group A Invasive Disease*

Group A streptococci (GAS) cause a wide spectrum of bacterial disease that ranges from noninvasive to life-threatening infections. NIAID-supported researchers developed a prototype vaccine that uses a recombinant protein containing protective fragments of six different M proteins. The M protein is one of the virulence factors produced by GAS. These researchers then evaluated different constructs and optimized the immune response to the vaccine. This important work has led to the first GAS vaccine clinical trial in 30 years. In addition, NIAID intramural scientists, using molecular genetic analysis combined with immunologic studies, implicated a 4-amino acid duplication in the extreme N terminus of M protein as a factor contributing to an epidemic wave of serotype M3 invasive infections. This finding has implications for GAS vaccine research. In another intramural study, NIAID scientists discovered a GAS mechanism to detect human innate host defense that triggers a pathogen survival response, in which cell wall synthesis is critical. These studies identify new potential vaccine antigens and targets for therapeutic interventions designed to control streptococcal infections.

*Streptoccus Group B*

Group B streptococci (GBS) cause serious bacterial illness in newborns, pregnant women, postpartum women, adults with chronic medical conditions, and the elderly. Nine serotypes of GBS have been identified, based on the capsular polysaccharide (CPS) of the bacteria. Because GBS CPS is the primary GBS virulence factor, vaccine development efforts have been focused on using GBS CPS to protect individuals from GBS disease. An effective vaccine will need to include the more virulent serotypes in the target population. Now, NIAID-supported researchers have developed an alternative method for GBS serotyping using techniques that do not require preparation of antisera. The new method was shown to be sensitive and specific when tested with GBS strains of all serotypes; it was also shown to be suitable for monitoring GBS isolates and could be applied in future epidemiologic studies to help in the development of GBS vaccines.

*Streptococcus Pnemoniae, Drug-resistant Invasive Disease*

From 2001 to 2004, levels of antibiotic resistance among pneumococci have remained relatively stable in adults, despite efforts at the national and local levels to reduce antibiotic overuse and promote greater use of new pneumococccal vaccine strategies. A new pediatric pneumococcal conjugate vaccine that has been shown to reduce carriage of specific serotypes of pneumococci in children was introduced in 2000. Use of the vaccine in children has been predicted to reduce rates of adult disease by reducing the reservoir of bacteria in children. Researchers have shown that levels of pneumococcal infection in adults due to serotypes contained in the new pediatric vaccine have dramatically declined over the last 3 years. To investigate this phenomenon further, NIAID-supported scientists developed a survey of adults to assess levels of pneumococcal vaccination of children in the home, where it appears likely that serotype replacement is leading to an increase in infection with nonpediatric pneumococcal serotypes in adults. While further work is needed to confirm these findings, the results suggest that future global vaccine strategies will need to

consider more strategic methods for targeting at-risk groups to preserve the efficacy of the existing vaccines as well as continued efforts to develop novel pneumococcal vaccines.

*Tickborne Encephalitis Virus*

One potential treatment for tickborne encephalitis virus (TBE) infection in humans is the administration of recombinant interferons. NIAID intramural scientists examined the JAK-STAT signal transduction pathway in TBE virus-infected cells following stimulation with interferon and found that virus infection of various cell lines and primary dendritic cells resulted in an inhibition of the phosphorylation of STAT1 and STAT2, a critical event for interferon responses. Dendritic cells are likely to be the first cell infected by TBE viruses and orchestrate the immune responses to virus infection. As phosphorylation of STAT1 is required for dendritic cell maturation, this work provides the first evidence of immune suppression by a flavivirus.

*Toxoplasmosis*

Infection with the common protozoan parasite, *Toxoplasma gondii*, causes a range of symptoms from asymptomatic chronic illness to mental retardation, retinal disease, and fatal brain infection. NIAID-supported investigators are studying the immune response to *T. gondii* to better characterize the basis for resistance to infection. NIAID-supported investigators demonstrated that mice lacking the gene for a factor involved in gene expression, STATl, were unable to control parasite replication and rapidly succumbed to the infection. By analyzing data from this study, these investigators showed that during toxoplasmosis the major role of STAT1 is not in the development of protective responses by immune system cells called T cells, but, rather in the development of other antimicrobial mechanisms. These results help elucidate the critical immunoregulatory effects of interferon-gamma (IFN-γ) in infections by intracellular pathogens and will improve the chances for development of anti-toxoplasma vaccines and drugs.

*Transmissible Spongiform Encephalopathies*

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases such as scrapie of sheep, Creutzfeldt-Jakob disease (CJD) of humans, bovine spongiform encephalopathy ("mad cow" disease), and chronic wasting disease (CWD) of deer and elk. TSEs are caused by accumulation of prion protein, an abnormal form of a protein found in humans and animals. Normal prion protein is expressed in a wide variety of tissues, yet conversion of normal prion protein to the TSE form appears to be restricted primarily to cells of the nervous and lymphoid systems. In order to determine why some cell types are more resistant to TSE infection than others, NIAID scientists developed a tissue culture system that allows them to monitor both acute and persistent abnormal prion protein (or PrP-res) formation. They demonstrated that, while any cell type can make new PrP-res following exposure to TSE infectivity, only some cell types go on to become chronically infected and make PrP-res persistently. This suggests that there are cell-specific factors that determine the susceptibility of a cell to chronic TSE infection. These factors, once identified, could be useful in designing effective anti-TSE therapeutics.

*West Nile Virus*

Over the past 3 years, NIAID has supported the preclinical development of a live, attenuated recombinant vaccine for West Nile virus (WNV). This vaccine was created by replacing several genes of the well-established yellow fever 17D vaccine virus with those of WNV. Preclinical testing of this WNV vaccine candidate (ChimeriVax-West Nile) has demonstrated safety, efficacy, and protection against disease in animal models (mice and non-human primates). Further development of this vaccine candidate, including phase I clinical trials in healthy adults, is under way. In addition, NIAID intramural scientists have developed two chimeric WNV vaccine candidates and one DNA vaccine candidate that have shown promising results in animal models. Despite the high level of attenuation, the chimeric vaccines induced moderate to high titers of neutralizing antibodies in monkeys and horses and prevented viremia in monkeys challenged with WNV. An Investigational New Drug application has been submitted to the Food and Drug Administration for the most attenuated of these two candidates.

The NIAID's VRC has developed a candidate vaccine for WNV using a codon-modified gene-based DNA plasmid vaccine platform to make DNA constructs that express WNV proteins. These vaccine constructs have undergone immunogenicity testing and viral challenge studies in mice. The VRC in collaboration with Vical, Inc., has completed GMP production of the vaccine for a phase I trial scheduled for early 2005.

NIAID-supported extramural scientists, using cryo-electron microscopy and image reconstruction techniques, were able to determine the strain of WNV responsible for the outbreak in the United States. Work is under way to increase resolution and obtain structures with more detail. Such detailed visualization and characterization of viral structure is expected to lead to the rational development of antivirals by identifying critical drug or antibody target sites that could disable virus infection or replication. In addition, recent studies from NIAID-supported investigators have produced the first high-resolution three-dimensional structure of an important functional domain of the WNV envelope protein. The envelope protein is the major surface protein of the virus and plays a central role in virus attachment and entry into cells. The envelope is also the primary target of protective host immunity against WNV infection. Determination of the WNV envelope protein structure may be useful in the identification of potential targets for the development of structure-based antiviral agents, immune-based therapeutics, or vaccines.

**Rare Primary Immunodeficiency Diseases**

*DiGeorge Syndrome*

Complete DiGeorge syndrome is a rare, fatal disorder resulting from congenital absence of the thymus, the organ responsible for the development of immune cells called T cells. Patients with this disease are profoundly immunodeficient. NIAID-supported researchers have previously shown that thymus transplantation is an effective therapy for this condition. These same researchers have now undertaken thymus transplantation in infants who have small numbers of functioning T cells prior to transplantation. Because there was no tissue matching between the donors and recipients, and even small numbers of T cells increase the risk of graft rejection, these patients were immunosuppressed with T-cell depleting agents prior to transplantation. The use of

T-cell depleting agents in other patient populations (e.g., kidney transplant recipients and cancer patients) has been associated with prolonged depression of T-cell counts lasting up to 1 year. In contrast, infants receiving a thymus transplant following T-cell depletion showed a return to baseline (subnormal) T-cell levels within 2 to 4 weeks, and within 6 to 7 months, the infants' immune systems achieved the same low-normal numbers of T cells as found in adults. Tests of T-cell function at the end of 1 year showed normal or greatly improved results as compared with the pretransplant period. These results demonstrate that host T cells can proliferate and mature in a nonmatched donor thymus, even after the use of T-cell depleting agents, and may have important implications for patients who require T-cell depleting therapies for a variety of diseases.

*Chronic Granulomatous Disease*

Chronic granulomatous disease (CGD) is an inherited disorder caused by a defect in an enzyme called phagocyte NADPH oxidase, or phox. Individuals stricken with CGD are prone to frequent, severe bacterial and fungal infections. In a study to assess the long-term clinical safety and efficacy of IFN-γ therapy, NIAID intramural scientists followed patients for up to 9 years and showed that long-term interferon prophylaxis is effective and well tolerated in patients with CGD.

**Autoimmune Diseases**

*Systemic Lupus Erythematosus*

Systemic lupus erythematosus (SLE) is a potentially life-threatening autoimmune disease in which the body attacks its own tissues and can harm the kidneys, lungs, central nervous system, and heart. Because autoantibodies are central to this damage, scientists believe that their development coincides with or precedes clinical disease. However, little is known about the autoimmune history of patients before SLE is clinically diagnosed. Recently, an NIAID-supported investigator and colleagues showed that clinical manifestations of SLE are preceded by autoimmune changes that are under way—and continue to progress—for many years before diagnosis. The study also provides a firm basis for the concept of using autoantibodies to diagnose lupus earlier, before symptoms appear, thereby enabling clinicians to offer treatment earlier in the disease course and improve treatment outcome.

An inflammatory mediator is crucial to the abnormal longevity of SLE T cells. Self-reactive antibodies are a hallmark of SLE, a prototypic and potentially fatal autoimmune disease that disproportionately affects women in their childbearing years. Production of these antibodies is driven by autoreactive T cells that are normally eliminated but persist in SLE patients. NIAID-supported investigators determined that comparison of the activities of thousands of genes in the T cells of SLE patients and normal controls showed that stimulatory conditions that caused normal T cells to be eliminated instead caused SLE T cells to increase their expression of cyclooxygenase-2 (COX-2) and survive. Moreover, inhibiting COX-2 activity abrogated the resistance of SLE T cells. The increased COX-2 was specific to SLE T cells and did not occur in T cells from other autoimmune diseases or cancer cells. Certain COX-2 inhibitors but not others were able to restore normal responses to SLE T cells, providing a rational basis for the use of these drugs for therapy and suggesting approaches to designing more potent and specific therapeutic drugs for use in SLE.

*Wegener's Granulomatosis*

Wegener's granulomatosis (WG) is an autoimmune disease that is characterized by the formation of chronic inflammatory lesions in blood vessels, resulting in vasculitis. Although this is a systemic disorder, it typically involves the lungs and upper respiratory tract. The cause of this disease is not yet known; however, it is believed that the inflammation results from the development of antibodies to proteins in the white blood cells that fight infection. Daily cyclophosphamide (CYC) and glucocorticoids have been found to be a highly effective treatment for WG. Unfortunately, long-term follow-up of patients treated with this regimen has shown that disease relapse is common and prolonged use of CYC can result in substantial long-term toxicity. For these reasons, NIAID intramural scientists have continued to search for less toxic yet efficacious treatment approaches that would be applicable to a wide range of patients. Mycophenolate mofetil (MMF) is an immunosuppressive agent currently used to prevent solid organ transplant rejection. MMF blocks the *de novo* purine synthesis in a pathway critical to normal function by white immune cells called lymphocytes. In a clinical study, MMF was well tolerated and no patients had to be withdrawn as a result of medication toxicity. MMF for remission maintenance was well tolerated and may represent a less toxic alternative to CYC for remission maintenance therapy in patients with WG.

**Other Rare Immune-mediated Conditions**

*Graft-versus-host Disease*

Chronic graft-versus-host disease (cGVHD) is the most common late complication of allogeneic bone marrow or peripheral blood stem cell transplantation and remains a significant barrier to more extensive application of transplantation in the treatment of cancers or blood disorders resulting from inherited immune deficiencies. In FY 2004, NIAID intramural scientists revealed that severe cGVHD is characterized by a phenotypic imbalance of a specific subset of T cells and showed that success in treating cGVHD is associated with progressive normalization of this imbalance. Thus, monitoring this subset of T cells may provide an important diagnostic tool to aid in the evaluation of new therapies to prevent or treat cGVHD.

NIAID-supported researchers discovered that a genetic variant of the interleukin 10 (IL-10) gene decreases the risk of acute GVHD and death after hematopoietic cell transplantation (HCT) by close to 50 percent. These findings provide avenues for development of novel IL10-based therapies to decrease GVHD and mortality in patients undergoing HCT and may help clinicians better inform their patients about relative risks of HCT. In another study, an NIAID-supported investigator developed an *in vitro* method for robust expansion of a specific type of immune cell called the T-regulatory cells ($T_{reg}$) and confirmed that these cells retain their immunomodulatory activity as evidenced by their ability to reverse new onset and chronic diabetes in a mouse model of type 1 diabetes and to prevent islet graft rejection in the diabetic mice. Most significantly, only very small numbers of $T_{regs}$ specifically reactive to a self-protein in autoimmune diabetes are needed to accomplish this reversal of disease. Expansion of $T_{regs}$ will allow further study of their characteristics, mechanisms of action, and application to other models of immune-mediated

diseases such as graft rejection and GVHD. Ultimately, *in vitro* $T_{reg}$ expansion and reinfusion into patients may be used to treat a variety of immune-mediated diseases.

*Hypereosinophilic Syndrome*

Hypereosinophilic syndrome (HES) is a rare and potentially fatal disorder. NIAID intramural scientists recently described a subset of patients with a myeloproliferative variant of hypereosinophilic syndrome (MHES) characterized by elevated serum tryptase levels, increased atypical mast cells in the bone marrow, tissue fibrosis, and the presence of a genetic abnormality in which two usually discrete genes are fused together causing an increase in activity of a particular enzyme. These scientists demonstrated that imatinib mesylate is effective and well tolerated in patients with MHES. Furthermore, the dramatic clinical and hematologic response to imatinib mesylate with molecular remission demonstrates that disease eradication may be an achievable goal. The dramatic resolution of myelofibrosis seen in patients with MHES treated with imatinib mesylate in the setting of a defined molecular defect also provides a unique opportunity to investigate the pathogenesis of the fibrotic response in this and other subgroups of patients.

**New Activities**

*Rare Infectious Diseases*

- Cytotoxic T-cell responses and T-helper responses appear to be critical components of immune responses to members of the flavivirus family. The Biodefense and Emerging Infections Research Resources Program has initiated the synthesis of overlapping peptide sets of five virion proteins of the WNV. The arrayed sets of 227 unique peptides will be made available to scientists conducting research on viral pathogenesis, immunological responses, and vaccine evaluation in a variety of animal models and humans.
- A randomized, double-blind phase I clinical trial is under way to study a treatment strategy called passive immunization in which human antibodies that can bind the WNV particles are injected directly into a patient's bloodstream. This ongoing trial was expanded from 35 sites to more than 60 sites in the United States and Canada.
- In FY 2004, NIAID funded 28 of more than 150 grant applications requesting funding for research on SARS.
- Through a grant supplement to the China CDC and their collaborators, NIAID initiated the development of three different SARS projects.
- NIAID awarded 2 contracts for SARS vaccine development; 2 contracts for antiviral screening against SARS; has evaluated more than 20,000 chemicals for anti-SARS-CoV activity; and has identified more than 1,400 compounds with activity against SARS-CoV.
- In FY2004, NIAID supported approximately 40 large-scale DNA sequencing genome projects for microbial pathogens and invertebrate vectors of infectious diseases.
- NIAID awarded five cooperative agreements in 2004 that are focused on GAS and GBS research in response to RFA 03-028, Partnerships for Vaccines and Diagnostic Development. Three of these grants are focused on the development of a GAS vaccine; one grant is focused on development of a GBS vaccine; and one grant is focused on development of an improved GBS diagnostic.

- Because of the increase in incidence of pertussis among neonates who are too young to have been immunized, a clinical trial began in the fall of 2004 to evaluate the safety of administering the pentavalent combination vaccine (DTaP-HepB-IPV; Pediarix) to infants at birth and 2 and 6 months of age along with other standard immunizations compared to the same pentavalent combination vaccine when given at 2, 4, and 6 months of age. Major objectives will include assessing the age-specific antibody responses following each vaccine dose and assessing T-cell and antigen presenting cell correlates of maturing immune responsiveness in neonates and young infants.
- In 2004, NIAID issued a renewal of the Tropical Diseases Research Units program (TDRU; http://grants2.nih.gov/grants/guide/rfa-files/RFA-AI-03-018.html. Three awards were made targeting cysteine proteases as antiparasitic targets and thymidylate synthase and fatty acid biosynthesis as antimalarial targets. The former project has led to the preclinical development of K777 as a possible oral treatment for Chagas' disease.
- In 2004, NIAID issued a renewal of the International Collaborations in Infectious Disease Research Program. The program currently involves 18 awards in 22 international sites.
- NIAID continues support of advanced development and manufacture of an MVA vaccine for smallpox, with the intention of targeting MVA vaccine candidates that can be produced at a scale to support commercial manufacturing. Bavarian Nordic and Acambis, Inc., have played a key role in the development of MVA vaccine candidates under contracts awarded in FY 2003. In FY 2004, new contracts were awarded to these two companies to build upon their achievements, focus on the manufacture of larger quantities of vaccine, and provide for safety and immunogenicity trials of the MVA vaccine candidates.
- NIAID continues support of advanced development and production of a recombinant protective antigen (rPA) vaccine for anthrax. In FY 2004, new contracts were awarded to Vaxgen and Avecia to build upon the companies' achievements, supporting production, testing, and evaluation of consistency lots, including a second phase II clinical trial.
- NIAID began construction of two integrated facilities that will include biocontainment laboratories: one on the NIH campus in Bethesda, Maryland, and one at the Rocky Mountain Laboratories in Hamilton, Montana. A third facility, which will be located in Frederick, Maryland, is planned.
- Through the *in vitro* and Animal Models for Emerging Infectious Diseases and Biodefense program, NIAID is screening existing FDA-approved antimicrobials and immunomodulators for efficacy against inhalational anthrax. Five licensed antibiotics have been selected for study, with ciprofloxacin as a control. NIAID also is pursuing studies to determine whether the course of antibiotic therapy can be decreased by vaccinating subjects with the rPA vaccine candidates currently under development.
- Through its *in vitro* and *in vivo* antiviral screening contracts, NIAID has supported the evaluation of hundreds of compounds for *in vitro* activity against models for hemorrhagic fever viruses such as yellow fever, Pichinde virus (a surrogate for Lassa), and Punta Toro virus (a surrogate for hantavirus). Approximately 240 compounds were screened in FY 2004.
- Over 1,500 compounds, including most of the licensed antivirals, have been evaluated for anti-poxvirus activities in cell culture. Those compounds that show activity *in vitro* have been tested in animal models (approximately 40). To augment the efficacy of cidofovir and its orally active derivatives, NIAID has fostered development of compounds with a mechanism of action different from cidofovir's via rational drug design and high-throughput screens. New targets for screens are being identified from genomic sequence information.

- An Investigational New Drug (IND) to support the use of cidofovir as primary treatment of smallpox has also been developed. Additional protocols are being written for special populations, including children and people with renal impairment. Since there has been no release of smallpox, no one has been eligible for this protocol.
- NIAID-supported Vaccine Treatment and Evaluation Units have developed clinical protocols to assess cidofovir as a treatment for complications related to smallpox vaccine. Cidofovir may be a viable back-up therapy after vaccine immune globulin has been indicated. Thus far, no one has needed to be enrolled in this protocol.
- The Food and Waterborne Diseases Integrated Research Network expands the NIAID's capacity to conduct research on food- and water-borne pathogens. Through the Microbiology and Botulism Research Unit, NIAID is funding the discovery of novel therapeutics to neutralize botulinum toxins in the blood or within the neuronal cell.
- The NIAID Small Business Biodefense Program (PAS-02-149) was created to support the development of therapeutics, vaccines, adjuvants/immunostimulants, diagnostics, and selected resources for biodefense. NIAID awarded 39 new biodefense grants to small businesses in FY 2004.
- NIAID awarded a contract to the Massachusetts Institute of Technology to support another Microbial Genome Sequencing Center to allow for rapid and cost-efficient production of high-quality, microbial genome sequences.

**Other Rare Immune-mediated Diseases**

- NIAID, in collaboration with the National Institute on Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Heart, Lung and Blood Institute (NHLBI), established the Clinical Trials in Organ Transplantation (RFA-AI-04-003) program**.** This clinical consortium was established to improve the success of transplants for end-stage organ disease, e.g., end-stage renal disease (ESRD). ESRD is a frequent complication of many autoimmune diseases. The goals of the consortium are to identify genetic factors in patients that could help doctors predict transplant outcomes as well as responses to post-transplant therapy; develop diagnostic tests that enable early detection and ongoing monitoring of immune-related processes; and test the safety and effectiveness of new, less toxic immunosuppressive drugs. The consortium is comprised of three institutions: Brigham and Women's Hospital, Boston; Cleveland Clinic Lerner College of Medicine of Case Western Reserve University; and the University of Pennsylvania, Philadelphia.

- NIAID established the Genomics of Transplantation Cooperative Research Program (RFA-AI-04-002) to support interdisciplinary, large-scale, broad-scope genomic studies in clinical transplantation, including solid organ, tissue, and cell transplantation. The goal of the program is to understand the genetic basis of immune-mediated graft rejection and differences in transplant outcomes and to provide a rational basis for the development of more effective treatment and prevention strategies to improve long-term graft survival and provide better quality of life for transplant recipients.

- The NIAID and NIDDK cosponsor the Clinical Islet Transplantation Consortium (RFAs DK-04-004 and DK-04-005) program to perform studies of islet transplantation in patients with type 1 diabetes to improve treatment of this disease. This consortium will develop and

implement single- and/or multi-center clinical studies, accompanied by mechanistic studies, in islet transplantation with or without accompanying kidney transplantation, for the treatment of type 1 diabetes.

- In 2004, NIAID funded five applications in response to RFA AI-03-019, Pathogenesis of Polyomavirus Associated Nephropathy, that are focused on basic, preclinical, clinical, and epidemiological research projects on polyomavirus-associated nephropathy (PVAN). PVAN is a serious, emerging complication in kidney transplant recipients. Research in this area will promote a better understanding of latent polyomavirus reactivation and virulence; enhance our knowledge of immune responses to polyomavirus infection associated with nephropathy; improve risk assessment for polyomavirus infection in transplant recipients; and stimulate the development of preventive, diagnostic, and treatment strategies for PVAN.

- The NIAID and the National Library of Medicine's National Center for Biotechnology Information launched the first public database of results from clinical blood and marrow stem cell transplants involving unrelated donors. Accessible at http://www.ncbi.nih.gov/mhc, this centralized resource provides genetic data as well as age, gender, and ethnicity information on more than 1,300 transplant donors and recipients from around the world.

**Ongoing Activities**

Rare Infectious Diseases

- NIAID continues to support invited investigator-initiated research grant applications focusing on the development of new diagnostics, prevention strategies, and treatments for toxins and pathogens listed in the NIAID Category A-C list of priority pathogens as well as the newly recognized SARS coronavirus.
- NIAID continues to collaborate with USAMRIID under an Inter-Agency Agreement to develop vaccine strategies for Ebola and other viral hemorrhagic fevers.
- NIAID and FDA, through an inter-agency agreement, continued to support the screening of compounds that may be effective against biodefense-related and emerging viruses, including vaccinia, cowpox, West Nile, yellow fever, and SARS, among others.
- NIAID supports the development of SARS coronavirus vaccines through a variety of grants and contracts.
- NIAID, in collaboration with USAMRIID and the Centers for Disease Control and Prevention (CDC), supports the *in vitro* screening of candidate drugs against the SARS coronavirus.
- NIAID continued its support for the Collaborative Antiviral Study Group (CASG), which is a collaborative network comprised of 63 institutions that conducts clinical studies of therapies for viral infections. Through the CASG, NIAID supports four pediatric clinical trials aimed at treating neonatal herpes simplex virus infections, sepsis caused by a group of viruses called enteroviruses, and cytomegalovirus (CMV) infections involving the central nervous system.
- NIAID continues to support a contract with the University of Alabama for a Respiratory Pathogens Reference Laboratory. This contract provides a resource facility with a major effort on reagent and assay development for measurement of the human immune response to targeted bacterial respiratory pathogens.

- NIAID continues to support phase I/II trials for two different candidate vaccines for human CMV. In one of the studies for which enrollment is ongoing, a vaccine against CMV is being evaluated in post-partum CMV-seronegative women for its ability to prevent infection in these women. In another study, four live recombinant viruses are being evaluated for safety in seropositive individuals. This vaccine was well tolerated with no significant side effects in this population.
- NIAID continues its involvement in a cooperative program with the USAMRIID to conduct mutually agreed upon research projects related to biodefense. Under this cooperative program, NIAID will provide funding to increase USAMRIID's aerosol exposure capability and expand appropriate containment facilities to better support studies to evaluate products for biodefense in non-human primates being conducted in the adjoining animal facility. Ongoing projects include developing vaccines for viral hemorrhagic fevers using Ebola virus as a model, and (in conjunction with FDA) evaluating the efficacy of antibiotic treatment in a model of pneumonic plague in African green monkeys. In conjunction with the National Cancer Institute, the F1–V Plague vaccine manufacturing process is being developed in order to provide material for further animal testing of the vaccine in both pneumonic and bubonic models of disease.
- NIAID continued its support of an ongoing phase III clinical trial conducted by the Adult AIDS Clinical Trials Group to determine whether the antiviral drug valganciclovir is safe and effective in preventing cytomegalovirus organ damage in HIV-infected subjects.
- NIAID has continued its support of the Bacteriology and Mycology Biostatistical and Operations Unit and the Bacteriology and Mycology Study Group initiatives, which support clinical trials against fungal and resistant bacterial infections.
- NIAID continues to support research on the prevention of GBS disease through a contract awarded to researchers at Brigham and Women's Hospital. This collaborative multidisciplinary effort is focused on clinical studies in selected populations to further understand GBS infection and on studies of the host immune response.
- Awards were made under the Partnerships for Vaccines and Diagnostic Development program, which focuses on the development of vaccines against GAS, GBS, and *Helicobacter pylori*.
- For over 30 years, NIAID has supported two helminth (parasitic worm) resources that serve the research community. The Schistosome Resource Center is maintained by the Biomedical Research Institute and the Filaria Resource Center is maintained by the University of Georgia.
- NIAID has continued to actively test new candidate compounds for efficacy against infectious complications of AIDS in culture and in animals through its anti-infective drug development contracts. These contracts have been awarded for research on several rare diseases caused by these microorganisms: *Mycobacterium avium, Pneumocystis, Cryptosporidium, Cryptococcus,* and *Microsporidium.*
- NIAID continued its support of research into the fundamental mechanisms of TSE disease and transmission as well as the development of diagnostic tests and effective therapies.
- NIAID has continued its support for the Pathogen Functional Genomics Resource Center at The Institute for Genomic Research (TIGR). The center was established to provide and distribute to the broader research community a wide range of genomic and related resources and technologies for the functional analysis of microbial pathogens and invertebrate vectors of infectious diseases.
- NIAID has continued to provide support for databases of genomic and postgenomic information and analysis tools on sexually transmitted pathogens and poxviruses.

- NIAID and the Department of Defense (DoD) are collaborating to provide for the coordinated development of a recombinant vaccine to protect against serotypes A and B of botulinum toxin. Through a Memorandum of Understanding (MOU) with DoD's Chemical/Biological Defense Program, NIAID is advancing the development of the Army's tularemia LVS vaccine by conducting toxicology testing and phase I clinical trials. The MOU is being extended beyond its original 2004 agreement to continue this collaboration.
- NIAID continues to participate in a coordinated Federal effort in biodefense genomics and is a major participant in the National Inter-Agency Genomics Sciences Coordinating Committee (NIGSCC) that includes many Federal agencies. NIGSCC funded the National Academies of Sciences to convene a committee, hold a workshop, and produce a report that analyzes the scientific issues that accompany the public release of genome sequences for infectious agents with potential national security implications.
- NIAID continues to coordinate genomic and postgenomic initiatives, including those related to biodefense, with other Federal agencies through participation in the Microbe Project, a Federal Interagency Working Group that coordinates microbial genomic activities. In addition, NIAID maintains contact with key scientists at specific agencies such as the Central Intelligence Agency (CIA), the Federal Bureau of Investigation (FBI), the FDA, the CDC, the DoD, the Department of Energy, the National Science Foundation, and the U.S. Department of Agriculture (http://www.ostp.gov/html/microbial/start.htm).

**Rare Primary Immunodeficiency Diseases**

- NIAID, along with the National Institute of Child Health and Human Development (NICHD), continue to support the Primary Immunodeficiency Diseases Consortium. The Consortium: (l) provides leadership and mentoring; facilitates collaborations; enhances coordination of research efforts; and solicits, reviews, recommends, and makes awards for pilot or small research projects; (2) maintains and expands a primary immunodeficiency diseases registry, which provides data to the research community about the clinical characteristics and prevalence of these diseases; and (3) develops a repository of specimens from subjects with primary immunodeficiency diseases. In the first year, the Primary Immunodeficiency Consortium awarded nine pilot research proposals.

**Autoimmune Diseases**

- NIAID, the NIDDK, and the NIH Office of Research on Women's Health (ORWH) continue to cosponsor the Autoimmunity Centers of Excellence (ACEs), a cooperative program that supports collaborative basic and clinical research on autoimmune diseases, including single-site or multisite pilot clinical trials of immunomodulatory therapies.
- The Autoimmune Disease Prevention Centers conduct basic research on the development of new targets and approaches to prevent autoimmune diseases and to evaluate these approaches in pilot and clinical studies. The Prevention Centers are cosponsored by NIAID, NIDDK, NICHD, ORWH, and the Juvenile Diabetes Research Foundation International (JDRF).
- NIAID supports the Multiple Autoimmune Diseases Genetics Consortium (MADGC), a repository of genetic and clinical data and specimens from families in which two or more individuals are affected by two or more distinct autoimmune diseases. This repository provides

well-characterized material for use in research aimed at identifying the genes involved in autoimmune diseases.

- Through the Stem Cell Transplantation for Autoimmune Diseases Consortium, NIAID is supporting clinical trials to assess the efficacy of hematopoietic stem cell transplantation to treat several severe autoimmune diseases, including multiple sclerosis, SLE, and scleroderma.
- NIAID chairs the NIH Autoimmune Diseases Coordinating Committee (ADCC). The ADCC was established in FY 1998 at the request of Congress to increase collaboration and facilitate coordination of research among NIH Institutes and Centers, other Federal agencies, and private groups interested in these diseases. The ADCC Autoimmune Diseases Research Plan, which was mandated in the Children's Health Act of 2000 (P.L. 106-310), was presented to Congress in FY 2003.

**Other Rare Immune-mediated Diseases**

- NIAID continues to support a multi-year cooperative agreement titled Systems Approaches to Innate Immunity, Inflammation, and Sepsis to a multidisciplinary team of researchers at the Scripps Research Institute. These researchers are employing a systems biology approach to create a comprehensive picture of innate immunity, an essential first line of defense against bacterial, viral, and fungal diseases.
- NIAID, NIDDK, and JDRF cosponsor the Immune Tolerance Network (ITN), an international consortium of scientists and clinicians dedicated to the clinical evaluation of promising tolerance induction therapies in four areas: autoimmune diseases, kidney transplantation, islet transplantation, and asthma and allergic diseases. The network is also developing assays and biomarkers to measure the induction, maintenance, and loss of immune tolerance in humans and is studying underlying mechanisms as an integral part of all clinical trials. The ITN includes basic scientists and physicians at more than 40 institutions in the United States, Canada, Europe, and Australia.
- NIAID continues to support the Hyperaccelerated Awards for Mechanisms in Immunomodulation Trials. This initiative supports immune-based mechanistic studies associated with clinical trials of infectious disease vaccines and immunotherapies for immune-mediated diseases. Applications are abbreviated, submitted and reviewed monthly, and awarded as early as 13 weeks after submission. This program is cosponsored by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), NIDDK, and the National Institute of Neurological Disorders and Stroke (NINDS).
- NIAID, in conjunction with NIAMS, NINDS, ORWH, and the National Multiple Sclerosis Society, continues to support the Sex-based Differences in the Immune Response research initiative. Differences in the immune response of males and females have been documented, and women are more likely than men to suffer from autoimmune diseases. The cause of pregnancy-induced changes in immune-mediated diseases and differences in the rate and severity of disease are unclear. An increased understanding of the mechanisms underlying the differences in the immune response in males and females should allow more targeted approaches for the prevention and treatment of immune-mediated disease.

**Rare Disease-specific Scientific Conferences, Symposia, and Meetings**

- NIAID convened an Expert Panel on Botulinum Therapeutics in February 2004 to identify the technical opportunities for development of novel post-exposure therapeutics for the botulinum neurotoxins. (http://www.niaid.nih.gov/biodefense/research/strat_plan.htm).
- NIAID led an Inter-agency Working Group on Recombinant Botulinum Toxin Vaccine Formulation in February 2004. An interim report was submitted to the Office of the Assistant Secretary for Public Health and Human Services, DHHS, in March 2004.
- A workshop was held in March 2004 to discuss prevention of GAS diseases and their sequelae with the focus on vaccine-related issues. This meeting was supported by the National Vaccine Program Office and included clinical investigators, research scientists, participants from U.S. government agencies (NIH, CDC, and FDA) and international organizations. Recommendations from the meeting focus on accelerating development of GAS vaccines and include the development of standardized protocols for surveillance, establishment of immune correlates of protection, considerations for vaccine trial design, and strategies for implementation.
- In March 2004, NIAID held a workshop to discuss the development of NPC-1161B, a drug administered orally to treat *Pneumocystis carinii* pneumonia (PCP). The meeting focused on several aspects comprising the critical path for clinical development, including good manufacturing practices (GMP) production, pharmacokinetics, toxicology, and planning of phase I clinical trial protocols.
- NIAID staff leads an Inter-agency Task Force to assess the vulnerability of specific foods to contamination with botulinum neurotoxins (March 2003 to present).
- On April 29–30, 2004, the NIAID and the Food Allergy and Anaphylaxis Network cosponsored the Symposium on the Definition and Management of Anaphylaxis. The meeting provided a forum for clinicians from different medical specialties to review the pathophysiology of anaphylaxis, compare the working definitions of anaphylaxis used by various clinical specialties, and discuss treatment options. The outcome of the symposium will be published for dissemination to the broader medical community and encourage ongoing dialogue that will lead to a more cohesive approach to diagnosis, prevention, and clinical management of anaphylaxis and to identification of promising directions for future research.
- The NIAID supported a workshop, titled Hematopoietic Stem Cell Expansion and Immune Reconstitution, held on May 17, 2004. The goals of the workshop were to evaluate the present knowledge of bone marrow transplantation as well as the future therapeutic value of *ex vivo* expanded cells in the context of a possible medical countermeasure against radiation resulting from a terrorist attack or accidental exposure to radiation or nuclear materials.
- On May 18–20, 2004, NIAID sponsored the 13th Annual International Centers for Tropical Diseases Research (ICTDR) Network meeting. The meeting included sessions on Progress in Tropical Vaccinology and Immunology, Epidemiology of Tropical Diseases, Progress in Pathogenesis Genetics and Genomics in Tropical Diseases, and Drug Development and Resistance.
- In May 2004, NIAID sponsored a workshop to address research on the control of arboviral encephalitides, including WNV. Numerous specific recommendations regarding research needs for alphavirus and flavivirus biology, diagnostics, vaccines, and therapeutics were generated by the group. These recommendations will be used to plan future activities and initiatives within NIAID/NIH.

- NIAID and the NIH Office of Rare Diseases cosponsored the second Federation of American Societies for Experimental Biology (FASEB) Summer Research Conference on Transplantation Immunology, which was held June 19–24, 2004, in Snowmass, Colorado. A central theme of the conference was that research in this field is expected to provide a new generation of tools for selective immunotherapeutic prevention and intervention, for example in the areas of bone marrow as well as solid organ transplantation.
- On September 14, 2004, NIAID convened a plenary meeting in Bethesda of principal investigators from grants funded under RFA AI-01-005, Sex-based Differences in the Immune Response, and representatives from the cosponsors, including NIAID, NIAMS, NINDS, and the National Multiple Sclerosis Society. Investigators presented updates on current research and discussed resources, collaborations, and future directions.
- NIAID sponsored a workshop on Eosinophilia-Myalgia Syndrome (EMS) on October 22, 2004. The meeting was attended by an expert panel, by members of the National Eosinophilia Myalgia Syndrome Network, and by several Federal agencies: FDA, CDC, and NIH Office of Dietary Supplements, NIAMS, and the National Institute of Environmental and Health Sciences. The meeting reviewed the epidemic of EMS in the late 1980s, summarized what has been learned, identified possible approaches to deal with future comparable epidemics, and discussed gaps in knowledge and directions for future research.
- In October 2003, NIAID cosponsored a meeting with the NIH Office of Rare Diseases on genomics and proteomics of rickettsial biodefense pathogens, including *Rickettsia rickettsii*.
- NIAID staff meet on a quarterly basis with staff from the DoD Joint Vaccine Acquisition Program (JVAP) to discuss and coordinate cross-cutting issues related to their respective development activities of a recombinant botulinum vaccine.
- NIAID staff led an Inter-agency Animal Models for Botulism Working Group.
- NIAID staff participated in the Department of Homeland Security (DHS) Bioshield Scenarios Workshop: Botulinum Toxin. This workshop supported DHS efforts to develop requirements for medical countermeasures for this toxin.
- NIAID established an Inter-agency Agreement with the DoD Armed Forces Research Institute of Medical Sciences (AFRIMS) site in Thailand to develop animal models of shigellosis, test shigella and other enteric vaccines in phase I trials, and gather data on endemic plague.
- NIAID is participating in the FBI-sponsored Scientific Working Group on Microbial Genetics and Forensics. Other participants include Federal agency officials and scientists with expertise in genomics, bioinformatics, microbiology, and infectious diseases. The working group's mission is to define criteria and coordinate the development and validation of microbial forensic methods that will support criminal investigations.

## NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES (NIAMS)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is to support research into the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases, the training of basic and clinical scientists to carry out this research, and the dissemination of information on research progress in these diseases. NIAMS-supported researchers have made significant progress in broadening the base of knowledge related to many of the rare diseases within the Institute's scope. Many of our new and ongoing basic and clinical research studies are aimed at reducing the burden associated with disease as well as the development of new treatment options.

### Recent Scientific Advances in Rare Diseases Research

*Epidermolysis Bullosa*

Epidermolysis bullosa is a group of hereditary rare blistering diseases of the skin. The disease is a result of defects in the type VII collagen, a molecule involved in anchoring the epidermis to the dermis. There has been much work investigating the use of gene therapy to provide a method to correct the collagen defect in epidermolysis bullosa. To date, while there has been some success in animal models, nothing has progressed to successful human clinical trials using this approach. NIAMS-supported researchers are exploring alternative methods of correcting the defect by providing the gene product, in this case normal human type VII collagen, rather than correcting the defective gene by introducing new genetic material, as with gene therapy. While this method would not allow for the development of the gene product naturally, it would provide a more physiologic method of trying to treat and heal the wounds that develop in patients with this disease. Using both human and mouse skin models, NIAMS-supported researchers injected human recombinant type VII collagen into the part of the skin where it is normally found in both models. Once injected into the right area, the collagen organized into its normal structure in the appropriate location and reversed the features of epidermolysis bullosa in both the human and animal skin models. This research provides a novel alternative to gene therapy for the treatment of the severe forms of this disease.

*Ichthyosis*

One of the primary functions of the outermost layer of skin is to act as a barrier both to loss of water and nutrients from within the body and to prevent foreign chemicals from entering the body. This function is accomplished by the outermost layer of skin called the stratum corneum. Ichthyosis is a heredity disease that has barrier function defects because of abnormalities in the stratum corneum. NIAMS-supported researchers have been investigating the molecular basis of the development of the barrier function in order to better understand the defects associated with ichthyosis. These researchers are examining a molecule involved in the production of a form of vitamin D in the skin and its role in the normal maturation of the skin and production of the barrier. To investigate the function of this molecule, researchers created a mouse model in which

the gene for this form of vitamin D was removed. In animals with only one rather than two genes for this form of vitamin D the skin looked normal. However, when assessed by a variety of biochemical measures and markers of stratum corneum function, it was noticed that the stratum corneum had decreased quantities of these molecules. When barrier function was tested by measuring water loss, it was found to be normal in the resting state but delayed in recovery following injury. Thus, this form of vitamin D is essential for the normal recovery of a barrier function following injury. Understanding the barrier function and its defects in certain skin diseases will allow for development of novel treatment options for individuals with ichthyosis and other diseases associated with abnormal barrier function.

*Juvenile Rheumatoid Arthritis*

Juvenile rheumatoid arthritis (JRA) is characterized by inflammation that causes redness, swelling, warmth, and soreness in the joints. Besides joint swelling, systemic JRA is characterized by fever and a light skin rash and may also affect internal organs such as the heart, liver, spleen, and lymph nodes. A genetic variation within the IL-6 gene increases susceptibility to systemic JRA, according to researchers funded by the NIAMS and the Arthritis Research Campaign. Researchers from around the world collaborated to collect DNA samples from children with systemic JRA and one or both parents. The transfer of genetic information from parent to child was analyzed, and the scientists found excess transmission of a genetic variation (-174G nucleotide variant) within the IL-6 gene from parent to child. Children who developed systemic JRA at age 5 or older showed significantly higher levels of this variant compared to the children who developed the disease before age 5. These findings suggest that there may be distinct genetic profiles for the disease that result in differences in age of onset and disease severity. Continuing to uncover disease-associated genes may lead to health care providers having clinically useful subgroupings of systemic JRA.

*Muscular Dystrophies*

Muscular dystrophy refers to a group of genetic diseases characterized by progressive weakness and degeneration of the skeletal or voluntary muscles, which control movement and breathing. The muscles of the heart and some other involuntary muscles are also affected in some forms of muscular dystrophy, and a few forms involve other organs as well. NIAMS-supported researchers have discovered and demonstrated in mice a method of delivering genetic therapy for muscular dystrophy and perhaps other diseases of the muscle or heart. Their research shows for the first time a method by which a corrected gene for dystrophin (a protein found in normal muscle tissue) can be delivered systemically to the affected muscles of a mouse with a disease that resembles Duchenne muscular dystrophy. Scientists found that by combining a single injection of an adeno-associated viral (AAV) vector (a viral “vehicle” carrying a mini-dystrophin gene) into the bloodstream, along with a dose of the hormone-like substance vascular endothelial growth factor, they were able to increase the efficiency of delivering the therapeutic virus into the muscle tissue. This treatment resulted in widespread expression of dystrophin in skeletal muscle tissue and improvement in several of the measurements of muscle integrity and function. These findings suggest an approach for overcoming one of the significant obstacles of gene therapy for muscular dystrophy, that of efficient delivery of therapeutic genes in the skeletal muscle cells. The Muscular Dystrophy Association also helped fund this research.

Researchers in the Intramural Research Program at NIAMS are exploring the development of potential treatments that would promote the regeneration of damaged muscle. These treatments, the researchers say, could be based on a protein called follistatin, which they have discovered plays a critical role in the growth and regeneration of adult skeletal muscle cells, and/or a group of chemicals called histone deacetylase (HDAC) inhibitors that help follistatin do its job. Researchers have found that the level of follistatin is significantly elevated in muscle cells when they are treated with HDAC inhibitors. HDAC inhibitors stimulate the formation of mature muscle cells from immature precursor cells. When follistatin levels are reduced, however, HDAC inhibitors no longer stimulate adult muscle growth, the researchers found. The regeneration activities of the HDAC inhibitors appear to function only in skeletal muscle, since follistatin is not stimulated in other cells tested. In animal studies, administering an HDAC inhibitor produced signs of muscle regeneration in regions of injured skeletal muscle tissue. This study establishes for the first time that follistatin promotes the recruitment and fusion of immature muscle cells to pre-existing adult muscle fibers.

*Osteogenesis Imperfecta*

Osteogenesis imperfecta (OI), also known as brittle bone disease, is a rare, inherited disorder in which bone is fragile and highly vulnerable to fracture. The vast majority of OI cases are due to mutations in a gene that produces type I collagen, a protein that forms a network of fibers in bone and provides much of the tissue's strength. Although gene therapy is likely to remain experimental for some time, the goal of being able to repair genetic mutations through gene therapy techniques is being explored. The challenge for researchers has been the ability to target the appropriate gene and insert new genetic material at the correct location on the type I collagen gene without affecting other unrelated genes. Using mouse models, NIAMS-supported researchers have been able to successfully insert the genetic material into the collagen gene. Importantly, cells in which the defective collagen gene was inactivated were shown to produce normal collagen and to retain the ability to develop into mature bone-forming cells.

**New/Planned Extramural and Intramural Research Initiatives**

*Juvenile Rheumatoid Arthritis*

NIAMS supports a state-of-the-art genomics project to uncover gene expression patterns that contribute to the development of pediatric arthritis. By using DNA microarrays—small silicon chips that contain tiny amounts of thousands of known genes—to carry out a technique called gene expression profiling, NIAMS-supported researchers will analyze thousands of genes in the blood, fluids, and tissues of children newly diagnosed with various types of pediatric rheumatic diseases. Identifying gene expression patterns—groups of genes that are "turned on"—for different types of childhood arthritis will help to improve diagnosis and to predict disease severity for affected children.

Two separate clinical trials are examining therapeutic interventions in children with JRA. One trial is investigating osteopenia (reduced bone mass), a frequent complication of JRA. This clinical trial measures the effectiveness of daily oral calcium supplementation to increase total body bone mineral density.

The long-term goal is to determine the safety and effectiveness of current and new biologic and pharmacologic treatments as alternative treatments to calcium in those JRA patients with osteopenia.

*Marfan Syndrome*

Marfan syndrome is an inherited disorder caused by a mutation in the fibrillin gene. This mutation causes the tendons, ligaments, and other connective tissues in the body to weaken. Marfan syndrome can affect the heart, skeletal system, eyes, and other organs in the body and symptoms range from mild to severe. The NIAMS recently awarded a program project grant to develop a multi-site translational research program in Marfan syndrome. The long-term goal of this program is to translate basic research in matrix biology into treatment strategies for individuals with Marfan syndrome and related disorders of connective tissue. The program will utilize a comprehensive and multidisciplinary approach that integrates the scientific interest and expertise of four leading laboratories in this and related research fields. Researchers will study genetically engineered mouse models of Marfan syndrome to uncover the abnormal cellular activities that contribute to this disorder and will translate this new knowledge into more effective therapies.

**Significant Ongoing Rare Diseases Research Initiatives**

*Scleroderma*

Scleroderma, often referred to as a single disease, is actually a symptom of a group of diseases that involves the abnormal growth of connective tissue, which supports the skin and internal organs. In some forms of scleroderma, hard, tight skin is the extent of the disease. In other forms, however, the problem goes much deeper, affecting blood vessels and internal organs such as heart, lungs, and kidneys. A NIAMS-funded project is using a unique sample set—lung tissue from scleroderma patients undergoing lung transplant surgery as well as lung tissue from unused donor lungs—to facilitate investigation into the cellular changes that cause the hardening of the lungs. Other NIAMS-supported researchers are examining the cellular and molecular processes of scleroderma, cell transfer between mother and child, and the development of innovative therapies.

*Juvenile Systemic Lupus Erythematosus*

In the area of childhood lupus, NIAMS-supported researchers are currently conducting a large, controlled study to assess the ability of statins (cholesterol-lowering agents) in preventing or delaying progression of cardiovascular disease in children with lupus. This research study involves 20 centers from the Pediatric Rheumatology Research Network in establishing the largest cohort of pediatric lupus patients ever prospectively studied. Approximately 15 percent of patients have been enrolled and baseline data analysis is currently under way.

*Muscular Dystrophies*

The NIAMS is collaborating with several other NIH components to boost muscular dystrophy research. Along with the National Institute of Neurological Disorders and Stroke (NINDS) and the National Institute of Child Health and Human Development (NICHD), the NIAMS is supporting one of three cooperative research centers focused on muscular dystrophy. NINDS and NICHD are also supporting one center each. Researchers at two of the centers are studying gene and/or stem cell therapies to treat muscle diseases, in particular Duchenne muscular dystrophy. A third center is examining skeletal muscle at the cellular and molecular levels to determine which factors might contribute to problems in the muscular dystrophies. This center is focusing on myotonic and facioscapulohumeral muscular dystrophies. Supplemental funding for these centers is being provided by the Muscular Dystrophy Association.

*Neonatal Onset Multisystem Inflammatory Disease*

Neonatal onset multisystem inflammatory disease (NOMID) is a rare, chronic, inflammatory disease that leads to major disability in affected children. Intramural researchers at the NIAMS have identified the genetic cause for this disease. Research suggests that a pro-inflammatory cytokine (a protein involved in the body's immune response), IL-1, could be contributing to the disease manifestations of NOMID. The NIAMS has initiated a multicenter study to evaluate the safety and efficacy of anakinra, a drug that blocks the activities of IL-1. Results in children with NOMID indicate that IL-1 blockade is highly efficacious in improving clinical signs and symptoms of this disease including fevers, rash, joint pain, headaches, and conjunctivitis (inflammation of the eye) and in lowering inflammatory markers of disease activity. In addition, anakinra crosses the blood brain barrier and has led to improvement in inflammation in the brain in a number of children.

*Vasculitis Clinical Research Consortium*

The NIAMS, in collaboration with the NIH Office of Rare Diseases and the National Center for Research Resources, is supporting the Vasculitis Clinical Research Consortium (VCRC), a part of the NIH Rare Diseases Clinical Research Network. Four centers comprise the VCRC and will serve as the focal points for vasculitis research, both domestically and internationally, for patients and researchers. One goal of the research is to develop new biomarkers of vasculitis disease activity.

**Rare Disease-specific Scientific Conferences, Symposia, and Meetings**

*Osteogenesis Imperfecta*

In collaboration with the NIH Office of Rare Diseases, the NIAMS provided support for the meeting, "Mild OI—Toward Better Understanding and Treatment." Osteogenesis imperfecta (OI) is a genetic disorder characterized by bones that break easily, often with little or no apparent cause. Many significant medical problems associated with mild OI, such as hearing loss, spinal involvement with compression fractures, and neurological impairment, may appear only in adult life. For patients with mild OI, the intensive, multi-disciplinary health care that has typically

focused on the more severe pediatric forms of OI is seldom available. This meeting brought together a group of international experts in the clinical care of individuals with OI to help identify the major health care issues that face people with mild OI, to better define the natural history of mild OI, and to examine the genetic components of this disease.

*Pseudoxanthoma Elasticum*

Pseudoxanthoma Elasticum (PXE) is a systemic inherited disorder that affects the elastic tissue in the skin, eyes, and cardiovascular system, and it can result in severe and even fatal problems in affected individuals. In the past several years significant progress has been made in the basic understanding of the genetics of PXE and the structural components affected by PXE. These advances, however, have highlighted a lack of information linking genetic and molecular advances and the development of PXE. In FY 2004, the NIAMS provided support for a meeting to bring together investigators from various fields of research to discuss how relevant advances in biology, metabolism, genetics, and epidemiology may help to bridge this information gap. The meeting was cosponsored by the NIH Office of Rare Diseases, the National Eye Institute, the National Human Genome Research Institute, and PXE International, a nonprofit foundation that advances research on PXE.

*Scleroderma*

The NIAMS recently provided support for the "International Workshop for Scleroderma Research." This workshop focused on basic research related to the pathogenesis of scleroderma. Scientific sessions covered autoimmunity, genetics, gene expression, vascular injury, animal models, fibrosis, and matrix metabolism. Clinically related areas such as novel therapeutics and development of measures of disease were also covered. The workshop brought together investigators in scleroderma from throughout the world along with prominent researchers in related disciplines.

Fibrosis, or skin thickening, is a feature of several rheumatic, musculoskeletal, and skin diseases, including scleroderma. The scientific meeting, "Pathogenic Mechanisms of Fibrosis: Search for Common Ground," brought together internationally recognized leaders and experts in the field of fibrosis to provide a forum for the cross-fertilization of basic science approaches and clinically relevant problems. The primary focus was on common mechanisms and pathways of fibrosis.

**Activities with Voluntary Rare Diseases Organizations to Stimulate Research**

**Health Partnership Program**

The NIAMS Health Partnership Program (HPP) has made significant steps in achieving the mission of understanding health disparities in minority populations and providing direction for improving the health status and health outcomes of those communities affected. The HPP is a community-based research initiative that operates through a collaborative effort between NIAMS and Washington, DC, area community partners. Through this partnership, initiated in February 2000 with the program, the HPP has established the NIAMS Community Health Center (CHC), which is located in a medically underserved minority community in Washington, DC. This site

serves as a focal point for many of the program's activities, including the clinical study, *The Natural History Study of Rheumatic Diseases in Minorities*. In order to extend services to other communities the NIAMS has recently started providing consultation services for patients with rheumatic disease in a separate clinic in southeast Washington, DC.

**Education Activities on Rare Diseases for the Researchers, Public, and the Health Care Providers Communities**

*Information Dissemination*

The NIAMS is committed to a comprehensive program of information dissemination to patients and to their health care providers. Research advances are of limited value if they never reach the arena of health care, and they miss the goal of improving public health for all Americans. To this end, the NIAMS has updated several publications on rare diseases, including booklets in its *Questions and Answers* series on *Behcet's disease* and *lichen sclerosis* and information packets on pemphigus and other blistering skin disorders, amyloidosis, polymyositis/dermatomyositis, and sweating disorders. In addition, the NIAMS has expanded its collection of Spanish language publications by adding *El Sindrome de Marfan* (Marfan syndrome).

*NIH Senior Health Web Site*

Through the NIH Osteoporosis and Related Bone Diseases National Resource Center, operated by the NIAMS, the Institute is currently developing a module on Paget's Disease for the NIH Senior Health Web site. This module will provide information on symptoms and complications, diagnosis, treatment, and research on this rare bone disease. The Institute is also updating two publications for health professionals working with patients who have osteogenesis imperfecta: *Therapeutic Strategies for Osteogenesis Imperfecta: A Guide for Physical Therapists and Occupational Therapists,* and *Osteogenesis Imperfecta: A Guide for Nurses*. These publications will be available in print and included on the Institute's pediatric rheumatology CD-ROM.

*Pediatric Rheumatology CD-ROM*

The NIAMS, in collaboration with the Arthritis Foundation, is producing a pediatric rheumatology CD-ROM to provide pediatric health professionals with access to the latest information on pediatric rheumatic diseases and to encourage early diagnosis and treatment. Disease topics include heritable disorders of connective tissue, scleroderma, and osteogenesis imperfecta.

## NATIONAL INSTITUTE OF BIOMEDICAL IMAGING AND BIOENGINEERING (NIBIB)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) is to improve human health by leading the development and accelerating the application of biomedical technologies. The Institute is committed to integrating the physical and engineering sciences with the life sciences to advance basic research and medical care. The Institute works to achieve this mission by supporting research that has broad applicability across disease or organ lines.

The NIBIB funds research on rare diseases through its extramural programs via grant solicitations, although the Institute's primary support of research is through unsolicited, investigator-initiated grant awards. Rare disease research funded in FY 2004 includes adrenal carcinoma, aortic valve disease, Barrett's syndrome, basal cell carcinoma, bladder cancer, bone dysplasia, brain tumor, congenital adrenal, congenital heart disorders, hyperplasia, Cushing's syndrome, cystic fibrosis, glaucoma, glioblastoma, Huntington's disease, macular degeneration, Marfan syndrome, Niemann-Pick disease, renal disease, respiratory distress syndrome, and sudden infant death syndrome.

Highlighted below are some of NIBIB's activities related to treating and preventing rare diseases and conditions that fall within the purview of the Institute's mission.

### Recent Scientific Advances in Rare Diseases Research

*Niemann-Pick Disease Type C*

Niemann-Pick disease type C (NP-C) is a genetic pediatric neurodegenerative disorder that causes progressive deterioration of the nervous system. This metabolic disorder leads to a series of neurological problems that are ultimately fatal. This disorder affects an estimated 500 children in the United States. The disease is autosomal recessive and is inherited when a child receives two mutant genes, one from each parent. In NP-C, cholesterol derived from low-density lipoprotein accumulates in cells of the brain, liver, spleen, lungs, and bone marrow. This leads to an enlarged spleen and liver, poor muscle control, impaired eye movements, slurred speech, and dementia. NIBIB researchers have recently used magnetic resonance imaging (MRI) techniques to generate quantifiable images and data to discriminate NP-C mice from normal mice. Researchers now plan to investigate the two organ systems that are most affected by the disease, the brain and liver. The goal of the research is to develop MRI methods that monitor the progression of NP-C and drug therapies in mice that can then be applied for use in human patients.

*Bladder Cancer*

Bladder cancer is the sixth most common cancer in the United States. Approximately 53,200 people are diagnosed with bladder cancer each year. Gene therapy, a technique for correcting defective genes responsible for disease development, has demonstrated some success in inhibiting bladder cancer tumor growth. The current method, intravesicular BCG therapy, augments local production of immune mediators of tumor clearance. However, BCG therapy has no effect on 20

percent of patients with bladder cancer. NIBIB researchers have found a less virulent gene vector, mycobacterium smegmatis, that can deliver eukaryotic expression plasmids to mammalian cells, and for the first time, can deliver plasmids expressing the green fluorescent protein from a eukaryotic promoter to macrophages. Researchers now plan to use this discovery to engineer a new anti-tumor therapy for bladder cancer that is safer and more efficient than the current method.

One of the greatest challenges to successful treatment of bladder cancer is early detection and staging. Optical coherence tomography (OCT), a noninvasive imaging device, is used for imaging of *in vivo* bladder tissue structure and blood flow simultaneously. Research has shown that OCT can distinguish between normal and cancerous bladder cells. NIBIB researchers are now developing an improved OCT imaging device that integrates arrays of optoelectronic sources, detectors, and micro electronic processing and control systems. This will allow for more rapid, high-quality imaging that can lead to improved diagnosis of bladder cancer.

*Glaucoma*

Glaucoma is the leading cause of blindness in the United States. It is a disease of the optic nerve, the part of the eye that carries images to the brain. When damage to the optic nerve fibers occurs, blind spots develop. Blindness can occur if the blind spots go undetected and significant damage to optic nerve occurs. However, medical advances have made it easier to diagnose and treat glaucoma. NIBIB researchers have developed new high-frequency ultrasound systems that provide new opportunities to advance tissue evaluation in the anterior segment of the eye. This technique allows research to develop a two-dimensional map of blood flow in the anterior segment of the eye, providing important information in the staging and diagnosis of glaucoma.

## NATIONAL CANCER INSTITUTE (NCI)

### Overview of Rare Diseases Research Activities

### Recent Scientific Advances in Rare Diseases Research (FY2004)

### A. Extramural Projects

*Mechanisms of Esophageal Carcinogenesis*

The annual incidence of esophageal cancers in the U.S. is about 14,000 new cases with a death rate of about 13,300 every year. Esophageal cancers are of two types: squamous and adenocarcinoma. Both types of esophageal cancer are common in the United States and worldwide and, in fact, adenocarcinoma of the esophagus is increasing at a fast rate. However, early diagnosis and meaningful therapeutic approaches remain challenging. The NCI has funded a P01 to the University of Pennsylvania (Dr. Anil Rustgi, PI) to understand the mechanisms for esophageal cancer development and progression and to translate these findings into the clinic. To that end, and as a reflection of the interrelated nature of the work by the different investigators supported by the P01, Drs. El-Deiry and Rustgi have found TRAIL, a natural molecule that causes cells to die. However, esophageal cancer cells are resistant to TRAIL and this has been found to be associated with the increased expression of another survival molecule called Bcl-xL, which nullifies the effect of TRAIL. They are embarking upon studies to inactivate Bcl-xL such that these cancer cells become sensitive to TRAIL and hope to use TRAIL in combination with the downregulation of Bcl-xL as novel therapy for esophageal cancer so as to improve prognosis and survival.

P01-CA-098101

"Mechanisms of Esophageal Carcinogenesis"
Anil K. Rustgi, M.D.
University of Pennsylvania
Philadelphia, PA

### Discovery of Genes Involved in Pancreatic Adenocarcinoma

The technologies of array comparative genomic hybridization on a cDNA microarray platform and bioinformatics tools have been optimized to characterize the pancreatic adenocarcinoma genome. Copy number alterations of amplified and deleted genes were defined in a panel of 24 pancreatic adenocarcinoma cell lines and 13 primary tumor specimens. A systematic prioritization scheme was used to select 64 focal minimal common regions of recurrent copy number changes. A complementary expression profile analysis of genes residing within these 64 prioritized minimal common regions identified a subset of candidate genes with statistically significant association between gene dosage and mRNA expression. The integration of this copy number data with expression profiles and other cancer database information provides for a highly efficient entry point for the discovery of genes involved in the pathogenesis of pancreatic adenocarcinoma.

Aguirre, A.J., et. al.,"High-resolution characterization of the pancreatic adenocarcinoma genome," *Proc. Natl. Acad. Sci.* USA 101: 9067-9072, 2004.

R01-CA-099041

"Genomic and Genetic Characterization of Amplicons in Glioblastoma Multiforme"
Lynda Chin, M.D.
Dana-Farber Cancer Institute
Boston, MA

**Transforming and Growth Suppressive Activities of the PAX3-FKHR Oncoprotein**

Alveolar rhabdomyosarcoma (ARMS) is a pediatric soft-tissue sarcoma associated with the skeletal muscle lineage. A 2:13 chromosomal translocation, which is an exchange of parts of chromosomes 2 and 13, occurs in most cases of ARMS. This exchange brings together the genes coding for the PAX3 and FKHR transcription factors in a manner that results in a novel fusion protein that is a potent transcriptional activator. The N-terminal PAX3 part of the fusion protein contains the DNA binding domain and the C-terminal FKHR part contains a transcription activation domain. Structure-function studies showed that there are two functionally separable domains (homeodomain and paired box) in the PAX3-FKHR fused protein. The homeodomain is associated with the transformation activity but not the growth suppression activity, whereas the paired box is partially associated with the growth suppression activity. These findings demonstrate that the transforming and growth suppressive activities of PAX3-FKHR are mediated by distinct functional domains that are likely to affect different downstream targets and pathways. These studies could lead to the definition of the downstream targets involved in PAX3-FKHR-mediated transformation and growth suppression.

Xia, S.J. and Barr, F.G., "Analysis of the transforming and growth suppressive activities of the PAX3-FKHR oncoprotein," *Oncogene* 23: 6864-6871, 2004

R01-CA-064202

"Studies of the t (2:13) of alveolar rhabdomyosarcoma"
Frederic G. Barr, M.D., Ph.D.
University of Pennsylvania
Philadelphia, PA

**Autocrine Motility Factor Signaling Enhances Pancreatic Cancer Metastasis**

Pancreatic cancer is an extremely aggressive cancer in which the cells are highly motile in both invasion and metastasis. Many different cell-signaling pathways at once are found to be aberrant in this cancer, making it unusually difficult to attack. A new study from NCI-supported researchers has shed light on an important pathway in pancreatic cancer that holds promise of an avenue for therapy. Autocrine motility factor/phosphoglucose isomerase (AMF/PGI) is a ubiquitous and multifunctional enzyme that plays a key role in glucose metabolism in the cell's cytoplasm and is a potent mitogen and motility-inducing factor in the extracellular milieu. Using a mouse model in

which human pancreatic cancer cells are implanted into the pancreas of immunodeficient mice, these investigators showed that only the cells that were engineered to express AMF formed large tumors that aggressively metastasized to the liver. They also demonstrated that AMF expression caused the loss of E-cadherin expression in these cells and, further, that the likely cause is the upregulation of a transcription factor, SNAIL, which acts to suppress E-cadherin expression. Because E-cadherin is the cell adhesion molecule that maintains normal tissue structure and keeps cells in an epithelial-type, nonmotile state, its loss (which is known to occur in pancreatic as well as many other motile cancer cells) due to AMF is significant. Given these results, this group sought to determine the pattern of AMF in human tumor samples and found that 11 out of 13 pancreatic cancers, and no normal tissue controls, were strongly positive for AMF protein. The indication that AMF regulates the acquisition of a motile, transformed phenotype through the well-known pathway of E-cadherin means that it could be a useful target for therapy or prevention.

"Autocrine Motility Factor Signaling Enhances Pancreatic Cancer Metastasis"
Tsutsumi, S. et al., November 2004, *Clinical Cancer Research* 10:7775-7784
http://clincancerres.aacrjournals.org/cgi/reprint/10/22/7775

R01-CA-051714

"Motility Factor Receptor"
Avraham Raz, Ph.D.
Wayne State University
Detroit, MI

**Thioredoxin Is Downstream of Smad7 in a Pathway That Promotes Growth and Suppresses Cisplatin-induced Apoptosis in Pancreatic Cancer**

Pancreatic cancer invades surrounding tissue and metastasizes extremely aggressively and also is exceptionally difficult to attack, due to a great extent to the fact that many different cell-signaling pathways at once are usually found to be aberrant in this cancer. NCI-supported researchers have now gained new insight into how signaling by the growth factor transforming growth factor beta (TGFβ) leads to enhanced malignancy in pancreatic cancer. Dysregulation of the TGFβ pathway is known to be a factor in this and many other cancers and is found in a majority of pancreatic cancer patients. TGFβ signals through the mediation of a set of proteins called Smads. The inhibitory Smad 7, frequently overexpressed in pancreatic cancer, leads to loss of TGFβ's cell growth-suppressing action. In this study, pancreatic cancer cells were engineered to overexpress Smad 7, and the genes whose transcription levels were altered were identified by differential display technology. Thioredoxin (TRX) (a regulator of activation of several transcription factors including AP1, NFκB, and p53) was found to be highly expressed in response to Smad 7 overexpression. They next used laser capture microdissection to isolate cells from human pancreatic cancer samples and found that in half the cancers, TRX expression was increased and corresponded with Smad 7 expression increases as well. When TRX was then inhibited in the Smad 7-overexpressing cells by antisense technology or biochemical inhibition, it attenuated the Smad 7-induced anchorage-independent growth. The apoptosis-inducing drug CDDP had little effect on Smad 7-overexpressing cells but killed them in the presence of TRX inhibitors. This effect appeared to be

related to the ability of the Smad 7-overexpressing cells to activate NFκB, a well-known signaling molecule in cellular stress response. These scientists then went on to demonstrate the downregulation of the apoptosis-inducing molecule ASK1 in Smad 7 cells, which TRX inactivates, and the enhanced expression of other apoptosis-resistance signaling pathways also. This demonstration that TRX is induced by Smad 7 expression in pancreatic cancer cells and that it then acts as the effector of several of the known outcomes of the loss of TGFβ tumor suppression offers several potential avenues for therapy by attacking already defined signaling molecules and pathways.

"Thioreduxin Is Downstream of Smad7 in a Pathway That Promotes Growth and Suppresses Cisplatin-Induced Apoptosis in Pancreatic Cancer" Arnold, N.B. et al., May 2004, *Cancer Research* 64:3599-3606 http://cancerres.aacrjournals.org/cgi/reprint/64/10/3599

R01-CA-075059

"Dysregulation of TGF Beta Action in Pancreatic Cancer"
Murray Korc, M.D.
Dartmouth College
Hanover, NH

**Kaposi's Sarcoma Herpesvirus (KSHV)/Human Herpesvirus 8 (HHV8)**

Kaposi's sarcoma (KS) is an aggressive and disseminated cancer of the lungs, gastrointestinal tract, and lymph nodes that is frequently seen in patients with HIV. KSHV/HHV8 virus is etiologically linked to all forms of KS, primary effusion lymphomas (PEL), and multicentric Castleman's disease (MCCD) and sometimes to post-transplant lymphoproliferative disorder (PTLD). Herpesvirus genomes contain a latent and lytic group of genes. Latent genes are few in number, maintain the viral genome in the host cell nucleus, and orchestrate replication of the viral episome during host cell division. Lytic genes become activated and replicate when internal and/or external signals are received by the host cell. Transcription and translation of lytic cycle genes leads to the production of viral offspring. Studies within the past several years by several NCI-supported investigators have shown that some latent viral proteins interfere with the immune system's ability to recognize and destroy infected cells by several mechanisms. One group of NCI-supported investigators studying the lytic proteins have recently discovered that most host cell gene expression is strongly inhibited 10–12 hours after the start of lytic infection, except for rare cellular genes that may play important roles in pathogenesis. These investigations are advancing our understanding of KS pathogenesis and oncogenic sequelae in KS-associated disease states like PEL, MCCD, and PTLD.

R01-CA-073506

"Herpesviral Gene Expression in Kaposi's Sarcoma"
Don E. Ganem
University of California
San Francisco, CA

**Aspirin May Reduce Risk of Hodgkin's Lymphoma**

In the first study to examine the association between nonsteroidal anti-inflammatory drugs (NSAIDs) and Hodgkin's lymphoma, scientists found regular aspirin use to be associated with a 40-percent decreased risk of the cancer compared to nonregular aspirin use. The population-based case-control study by Ellen Chang, Sc.D., and Nancy Mueller, Sc.D., of Harvard School of Public Health, and colleagues compared data on 565 patients with Hodgkin's lymphoma and 679 controls. A reduction in risk was not observed with regular use of other NSAIDs. However, regular acetaminophen use was associated with a 70-percent increased risk of Hodgkin's lymphoma. Regular analgesic use was defined as having taken at least two tablets per week on average over the preceding 5 years. Dose-response relationships also were seen. Aspirin inhibits the transcription factor B (NF-B), which is involved in immune and inflammatory responses and which, in laboratory studies, appears to be critical in survival of Hodgkin's lymphoma cells. Perhaps aspirin guards against the cancer in this way.

Chang ET, Zheng T, Weir EG, Borowitz M, Mann RB, Spiegelman D, Mueller NE. Aspirin and the risk of Hodgkin's lymphoma in a population-based case-control study. *J Natl Cancer Inst* 2004 Feb 18; 96(4):305-15.

PubMed Link: http://jncicancerspectrum.oupjournals.org/cgi/content/full/jnci;96/4/305

**Dexrazoxane Protective against Heart Disease in Childhood Cancer Survivors**

Dexrazoxane (Zinecard), a free-radical scavenger that protects adults who receive anthracycline from developing heart problems, also may protect the heart from damage associated with doxorubicin chemotherapy. Doxorubicin is the most effective treatment for children with acute lymphoblastic leukemia (ALL), the most common malignancy in children, but it also injures myocardial cells. Many childhood cancer survivors experience late effects such as congestive heart failure and have an increased risk of death—sometimes sudden—from cardiac causes. These negative consequences are related, in part, to the toxic effects of doxorubicin and other cancer treatments.

In a study led by Dr. Steven Lipshultz of the University of Miami of more than 200 children newly diagnosed with high-risk ALL enrolled in a multicenter, randomized, controlled trial conducted at the Dana-Farber Cancer Institute in Boston, dexrazoxane was associated with a large and statistically significant reduction in the incidence of myocardial injury, and it did not compromise the anti-leukemic efficacy of doxorubicin, at least in the short term.

The study, published in *The New England Journal of Medicine*, reports that half of the children were treated using the standard multi-agent protocol for ALL, which includes doxorubicin. The others were treated with an infusion of dexrazoxane 30 minutes before receiving doxorubicin. The researchers used the children's troponin T blood levels during therapy to measure the impact on the cardiovascular system. Troponins are sensitive and specific biomarkers for cardiac injury. Half of

the children in the chemotherapy-only arm of the study had elevated troponin T levels, while only 21 percent of the children who received dexrazoxane showed an increase. These findings suggest that doxorubicin-associated myocardial injury in children may be, at least in part, related to oxidative damage. This is an important clue to the pathogenesis of this late effect. Longer follow–up will be needed to determine the long-term influence of dexrazoxane on cardiac function and event-free survival. The optimal dose of dexrazoxane is unknown, but the implications of this study prove that it is possible to increase survival while also reducing the morbidity associated with treatment.

The study was supported, in part, by funding from NCI's 1998 RFA to support research on long-term cancer survivors. That RFA was reissued, and NCI's Office of Cancer Survivorship recently awarded 17 new grants to continue research in this important area of scientific inquiry.

Lipshultz SE, Rifai N, Dalton VM, Levy DE, Silverman LB, Lipsitz SR, et al., the effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. *N Engl J Med*. 2004 Jul 8; 351(2):145-53.

PubMed:http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15247354
Cancer Bulletin Article: http://cancer.gov/NCICancerBulletin/NCI_Cancer_Bulletin_071304.pdf

**Development of Proteasome Inhibitor as a Therapy of Multiple Myeloma**

Based on initial animal model studies and a phase I trial, the protease inhibitor Bortezomib can overcome resistance to conventional therapy for multiple myeloma. This led to a multicenter phase II trial of Bortezomib in 202 patients, which demonstrated that 35 percent of patients had complete responses. These responses had median duration of 12 months, with survival of 17 months rather than the expected 6–9 months. The associated clinical benefit included increased hemoglobin and decreased transfusions, improved quality of life, improved normal immunoglobulins, and stabilization or improvement in renal function. This trial led to the FDA approval of this novel agent for treatment of relapsed refractory multiple myeloma. Subsequent gene profiling and proteomic studies have provided the basis for combining Bortezomib with conventional therapies (DNA damaging agents) or novel agents (hsp 90 inhibitors, histone deacetylase inhibitors, immunomodulatory drugs) in ongoing clinical trials. From our correlative studies of patients on clinical protocols we have identified mechanisms of resistance to Bortezomib (induction of hsp 27) that can be successfully overcome with p38MAPK inhibitors. Bortezomib therefore represents a first in class novel targeted therapy that can overcome drug resistance and has great promise to improve patient outcome in multiple myeloma.

**New Approaches to the Treatment of Patients with Metastatic Melanoma**

In this phase I/II clinical trial, the safety and efficacy of BAY 43-9006 in combination with chemotherapy was evaluated. In the study of 35 patients with metastatic melanoma, patients were treated with an oral drug BAY 43-9006 in combination with chemotherapy. BAY 43-9006 is a small molecular inhibitor of several kinases including the raf kinase. In addition, there are indications that this drug also inhibits angiogenesis. Encouraging results have been seen, with 40

percent of patients exhibiting a partial response and the majority of patients showing stabilization of disease. This is a significant achievement in patients with metastatic melanoma in which no therapy has been shown to alter survival. Additional laboratory correlative studies have shown that BAY 43-9006 does inhibit the molecular target BRAF. Overall, these preliminary studies show that a pill combined with chemotherapy shows significant antitumor activity in patients with metastatic melanoma. This unique approach to melanoma cancer treatment may be the first real advance in the treatment of patients with advanced melanoma.

**Single Agent CEP-701, a Novel FLT3 Inhibitor, Shows Biologic Response and Clinical Activity in Patients with Relapsed or Refractory Acute Myeloid Leukemia**

A clinical trial of CEP-701 was conducted at the Johns Hopkins and M. D. Anderson Cancer Centers in adult patients with relapsed and refractory acute myeloid leukemia (AML) with FLT3 mutations. The patients were treated with CEP-701 alone, which they took orally twice a day. Tests were developed to enable us to determine the level of FLT3 inhibition we were attaining in the patients. In the first 3 patients, inadequate FLT3 inhibition (<90 percent) was seen, therefore, the dose of CEP-701 was increased and clinical responses of decreasing leukemia cells were seen in 5 out of 14 patients. What is learned from experiments performed on the leukemia cells and plasma samples from the patients on treatment is that drug levels achieving >90-percent inhibition of FLT3 will demonstrate a decrease in leukemic cells. This study is the first to show that AML patients with FLT3 mutations have clinical responses to FLT3 inhibition and validates this as a target for AML treatment. These studies should encourage companies that have FLT3 inhibitors in development. The lack of response from solely using this inhibitor suggests that the next stage of clinical trial might be a combination of FLT3 inhibitors with chemotherapy.

**Phosphatidylinositol Signaling Cascade as a Therapeutic Target in Ovarian Cancer**

Vascular endothelial growth factor (VEGF) contributes to neovascularization and to the accumulation of ascites in the peritoneal cavity. Ascites accumulation remains a major cause of morbidity in ovarian cancer. Current study has looked at the effect of immuneutralization of VEGF with a VEGF-trap with and without taxol. Results from mice studies showed virtually no ascites developed in the combined treatment group or group treated with the VEGF-trap alone. Paclitaxel alone reduced ascites by 86 percent compared to controls. In the VEGF-trap plus paclitaxel group, morphologic studies demonstrated that most of the residual tumor showed degenerative changes, apoptosis, and necrosis. In addition, mice treated with the VEGF-trap plus paclitaxel showed no observable side effects; their health and behavior were indistinguishable from normal, non-inoculated mice. These data suggest that combination therapy with VEGF-trap plus paclitaxel may provide a novel therapeutic strategy for treatment of patients with ovarian cancer associated with ascites.

**Cross-species Approach to Target Metastasis Regulators**

Genetic manipulation of lower organisms has enabled researchers to identify molecules that control basic biological processes in humans and that contribute to disease pathogenesis. During normal development of the Drosophila ovary, a dynamic process called border cell migration occurs that resembles the metastatic behavior of human ovarian cancer cells. The approach is to

study human homologs of Drosophila genes to gain new insight into the migratory behavior of ovarian cancer cells. Myosin VI is absent from normal human ovary but is expressed in ovarian cancers at levels that strongly correlate with the aggressiveness of their clinical behavior. Inhibiting myosin VI expression in high-grade ovarian carcinoma cells by using antisense sequences and short interfering RNAs that specifically target the myosin VI gene was found to inhibit the ability of cells to spread and migrate *in vitro*. In subsequent experiments, researchers investigated the effect of inhibiting myosin VI on ovarian cancer dissemination in a mouse model. By imaging fluorescently labeled human ovarian cancer cells propagated in mice, it was observed that inhibiting myosin VI expression substantially impeded intraperitoneal spread of tumor cells. These studies indicate that (1) myosin VI is a potentially useful prognostic marker of ovarian cancer and (2) inhibiting myosin VI represents a novel approach for controlling dissemination of ovarian cancer.

**Brain Cancer Clinical Trials**

The Pediatric Brain Tumor Consortium, which currently has 10 active clinical trials, is studying molecularly targeted agents, including angiogenesis inhibitors, farnesyltransferase inhibitors, inhibitors of drug resistance, and inhibitors of epidermal growth factor signaling. These NCI-supported phase I trials provide the basis for the phase II and phase III studies of molecularly targeted agents that will define their role in the treatment of children with cancer.

NCI also supports clinical trials that specifically include children with cancers associated with neurofibromatosis-1 (NF1) through the pediatric clinical trials cooperative group. Of special relevance are the brain tumors associated with NF1 and in particular the low-grade gliomas that develop in these children. The Children's Oncology Group will complete accrual to its clinical trial for children younger than 10 years of age with progressive low-grade astrocytoma by December 2004. Approximately 400 children have now been entered into this study, including 115 children known to have NF1. For children with NF1, the primary objective of the study is to determine their event-free survival and overall survival following treatment with a regimen of carboplatin and vincristine. Accrual is limited to children with disease that is progressive after surgery or those whose risk of neurologic impairment with progression is high enough to require immediate treatment. Preliminary results from the study should be available in approximately 1 year.

**Clinical Development of Agents for Kidney Cancer**

Renal cancer is made up of several distinct cellular and genetic subtypes. The most common form of renal cancer, clear cell carcinoma, is related to loss of function of the tumor suppressor, von Hippel-Lindau (VHL). The absence of the functional protein product of the VHL gene leads to activation of several genes, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and other mediators of tumor angiogenesis, growth, and metastasis. The identification of molecular targets in the VHL pathway as well as other pathways relevant to kidney cancer (Ras-Raf-MEK-ERK and EGFR-MAPK-PI3K-Akt-mTOR), have led to partnerships with industries for the clinical development of targeted agents in kidney cancer.

- Promising results from a small trial of a monoclonal antibody that inhibits VEGF (Avastin®, bevacizumab, Genentech, Inc.) conducted at the NCI intramural program have led to a national trial through the cooperative groups and the Cancer Trial Support Unit (CTSU). The trial (CALGB 90206) opened in October 2003 and is accruing more quickly than anticipated—the targeted enrollment of 700 patients is expected by mid-2005. Questions such as whether interrupting VEGF signaling will lead to prolongation in survival will be answered, in addition to adjunctive studies addressing whether pre- and post-treatment plasma, urine, and/or baseline tumor tissue can select for patients likely to respond to anti-angiogenesis treatment.

- As of September 2004, 33 letters of intent have been received to study agents in renal cancer; more than 13 new studies have been approved. Agents under study include inhibitors of VEGF, PDGF, HSP-90 (stabilizer of HIF and EGFR), m-TOR, and raf kinase. Other novel agents include SB-715992 (ksp inhibitor), epothilone B, and SAHA. Many of these clinical trials include companion translational research, facilitating bidirectional bench-to-bedside research to accelerate progress in renal cancer.

- Combinations of targeted agents were the subject of a retreat of thought leaders conducted by DCTD as part of the new Critical Molecular Pathways initiative. This led to a number of pilots, including a formal solicitation in July 2004 for combinations of targeted agents in renal cancer. Interest in combination therapy in renal cancer is supported by preclinical and clinical data, e.g., a phase II study in renal cancer suggesting synergy between a VEGF and EGFR inhibitor (bevacizumab and erlotinib). Intellectual property, liability, and regulatory issues can slow progress in developing combination regimens if more than one company is involved. The NCI has played an important role in facilitating collaborations within the private sector without the need for additional bargaining between the parties. The NCI developed standard language now used in all agreements with industry concerning how data are to be shared and how companies may benefit from any invention that may arise using drug combinations.

- Variants of kidney cancer other than clear cell have distinct genetic profiles, comprise approximately 15 percent of diagnoses, and are more difficult to study due to their rarity. To find treatments relevant to these subtypes, three trials have opened nationally in the cooperative groups in papillary, collecting duct, and sarcomatoid renal cancers.

- Novel dendritic cell-based tumor immunotherapy is a very promising approach for cancer treatment. NCI has just funded a phase II clinical trial for treating renal cell carcinoma by dendritoma vaccine. The novelty of this trial is that dendritoma vaccine is made from patient's own tumor cells, dendritic cells, and autologous serum. This vaccine has been very effective in activating patient's functional cytotoxic T lymphocytes that efficiently lyse autologous tumor cells and reduce tumor burden. A total of 29 advanced renal cell cancer patients who have exhausted all available therapies will be recruited and evaluated in this trial, and correlative studies will be conducted to understand the molecular mechanisms of tumor-specific immune responses and treatment effects.

**Multiple Chemotherapy Drugs for Adjuvant Bladder Cancer Therapy**

CALGB 90104 is a new, randomized trial open to investigators nationally through the CTSU. It builds on a large, randomized trial of adjuvant therapy for breast cancer sponsored by NCI, in which patients received multiple chemotherapy drugs, with different mechanisms of action, in a particular sequence. Breast cancer patients receiving chemotherapy in this sequence had improved disease-free survival and overall survival. CALGB 90104 will attempt to extend this observation to the adjuvant therapy of bladder cancer by testing doxorubicin and gemcitabine followed by paclitaxel and cisplatin versus adjuvant cisplatin and gemcitabine.

**Gemcitabine for Bladder Cancer**

A national phase III trial is under development to test whether the immediate instillation of gemcitabine post-surgically in patients with newly diagnosed or occasionally recurrent superficial bladder cancer is better than the instillation of saline.

**The Chronic Lymphocytic Leukemia (CLL) Research Consortium**

Since its initial funding in 1999, the CLL Research Consortium has made substantial progress in research on the genetics, biochemistry, and immunology of CLL and has identified promising candidate therapies for this disease. Accomplishments over the past period of funding include:

***identification of***

- new genetic lesions involved in pathogenesis
- new mouse model for CLL
- novel mechanisms regulating cell death in CLL
- small molecule inhibitors of anti-apoptotic proteins
- accessory cells involved in leukemia pathogenesis
- leukemia-cell clearance mechanisms involved in cellular and immune gene therapies
- leukemia-associated antigens—one seminal paper showed ZAP-70 (a tyrosine kinase) expression is a stronger predictor of the need for early treatment of patients with CLL than is immunoglobulin mutation status (*NEJM* 351:893-901, 2004)
- novel pharmacologic and biologic agents for CLL

***improved understanding of***

- the state of T-cell anergy in CLL
- factors associated with disease heterogeneity

***development of***

- improved algorithms for assessing disease progression risk and response to therapy
- infrastructure that facilitates bench-to-bedside and bedside-to-bench research
- a Web-based biomedical informatics system
- a national tissue bank

Investigators of the CLL Consortium continue to interact with the NCI intramural investigators in familial CLL studies. They are accruing families with two or more living cases of CLL

(http://dceg.cancer.gov/fam-cllsi.html). These families provide material for genetic studies and for studies to characterize B-cell abnormalities that may be precursor states for CLL. Studies at NCI and elsewhere have found that first-degree relatives of CLL cases from high-risk families have a higher probability of developing monoclonal B-cell lymphocytosis than do individuals from the population at large. Members of the international familial CLL consortium have submitted a paper proposing standards and criteria for B-cell findings that can be applied to both population and high-risk family studies.

NCI investigators are collaborating with investigators at some of the CLL Research Consortium centers to standardize familial CLL studies. This involves designing a risk factor questionnaire to be used by all participating groups, developing a standard immunophenotyping protocol, and planning for enhanced family accrual and biospecimen collections so that new genome-wide scans can be conducted on CLL family samples pooled from multiple centers.

The CLL Research Consortium had its annual meeting in Bethesda, MD, in April 2004. Members of the public, professional societies, and advocacy groups as well as staff of NCI and NHLBI were invited to attend.

In August 2004, in the *New England Journal of Medicine*, the CLL Research Consortium reported that the level of ZAP-70, a tyrosine kinase, in leukemic cells correlated best with the median time from diagnosis to treatment. Determination of ZAP-70 may become a standard prognostic indicator in patients with CLL. This is important because differences in the clinical course of CLL make decisions about treatment difficult.

The CLL Research Consortium's competitive renewal grant application was reviewed in October 2004. Cytogenetic studies that take advantage of newly discovered genetic lesions of CLL and enhanced drug discovery studies based on molecular pathways in the pathogenesis of CLL are highlights of this competitive renewal application.

**Agents in the Drug Development Pipeline for CLL**

Additional research supported by NCI includes basic, preclinical, and clinical studies in multiple areas related to CLL. Promising agents that have shown activity in CLL include flavopiridol, clofarabine, IDEC-152 (anti-CD23 antibody), bortezomib (Velcade), epothilone-B, LMB-2, thalidomide, triapine, pentostatin, denileukin diftitox, and bryostatin.

**Liver Cancer**

The NCI continues to collaborate with NIDDK and the other NIH Institutes and Cancer Centers to sponsor research activities to prevent, diagnose, and treat liver cancer and to promote scientific conferences to exchange information about liver cancer. Over 90 percent of primary carcinomas of the liver are hepatocellular carcinomas (HCC). While HCC is a common cause of cancer and cancer-related mortality worldwide, until recently it has been considered a rare cancer in the United States. New studies demonstrate that liver cancer is the most rapidly rising cause of cancer in the United States, resulting in at least 14,000 deaths annually and ranking as the eighth most common cause of cancer death in men. The recent upsurge in HCC in the United States may be

attributable to chronic infection with hepatitis C virus (HCV), which is found in more than half of patients diagnosed with HCC.

This rise in the incidence of HCC has not been accompanied by improvements in early detection or treatment that might lead to improved patient survival. Liver cancer remains a highly fatal disease with average survival rates after the onset of symptoms of less than 1 year. The rising incidence of liver cancer, its continued high mortality rate, and the lack of effective treatments underlie the need for research into the etiology of HCC critical for the development of early detection methods and interventions that might counteract these trends.

**Experts Conference on Liver Cancer**

To help define the most urgent areas requiring additional research, the NCI cosponsored an Experts Conference with NIDDK on liver cancer. This conference, "Hepatocellular Carcinoma: Screening, Diagnosis, and Management," was held on April 2004 at the NIH. The key topics and summary recommendations from the conference were published in a special supplement to the November 2004 issue of *Gastroenterology* (Reference: *Gastroenterology* November 2004; 127(5): Suppl 1: S1-S323).

The summary recommendations focused on the promotion of research in four main areas: surveillance, prevention, early detection, and treatment.

- For surveillance, a major recommendation is to establish prospective databases of patients with HCC that could provide detailed information on the underlying risk factors for liver disease as well as serum and tissue samples for molecular analyses to further elucidate the pathogenesis of liver cancer and identify biomarkers of HCC risk and diagnosis that might serve as targets of therapeutic interventions.

- For prevention, the recommendations support research into strategies for primary prevention (public health initiatives to prevent viral hepatitis and other causes of liver disease), secondary prevention (improvement in therapies to treat liver disease), and tertiary prevention (evaluation of hepatoprotective agents and long-term antiviral suppressive therapy to prevent HCC in patients with cirrhosis).

- For early detection, the recommendations support research for the development of better biomarkers and/or more practical and sensitive imaging methods to detect HCC in high-risk patients as well as epidemiological studies to better define which high-risk individuals warrant active surveillance.

- For treatment, the recommendations support development and evaluation of innovative local ablative therapies, cytotoxic agents, and molecularly targeted therapies along with basic research to define key molecular pathways that contribute to the malignant transformation of liver cells.

Based on the recommendations from this conference, NCI and NIDDK are discussing the potential development of a Request for Applications specifically designed to address the main areas of research outlined above.

**Liver Cancer Research Initiatives**

The NCI also continues to be actively involved in the Liver Cancer Working Group included in the Action Plan for Liver Disease Research that is being developed by NIDDK and that will enable trans-NIH and trans-agency coordination of research initiatives in this area. In addition, NCI continues to support other research initiatives related to the surveillance, prevention, early detection, and treatment of liver cancer by outside investigators through research grants to academic institutions and by intramural, government scientists at the NIH.

The NCI cosponsored with NIDDK the first 5 years of the 7-year, randomized clinical trial called HALT-C trial (Hepatitis C Antiviral Long-Term Treatment against Cirrhosis). The HALT-C study is designed to determine if continuing interferon treatment over several years will suppress HCV, prevent progression to cirrhosis, prevent liver cancer, and reduce the need for liver transplantation.

**Clinical Trials for Lung Cancer**

The NCI is currently sponsoring development of 16 new investigational agents in 28 phase I and phase II clinical trials for patients with liver cancer that are being conducted by the extramural scientific community as well as a multi-institutional phase II trial evaluating radiofrequency ablation in patients with HCC.

**Hepatitis C and Liver Cancer**

In addition, NCI has recently awarded three grants to extramural investigators related to a multi-institute RFA on “Hepatitis C: Natural History, Pathogenesis, Therapy, and Prevention” that will facilitate our understanding of the mechanisms of liver cancer associated with viral infection and may provide new diagnostic and prognostic biomarkers and targets for therapeutic interventions in high-risk patients.

The main areas of basic HCC research for the intramural investigators at NIH include the etiology and molecular pathogenesis of HCC and molecular profiling and diagnosis. A major focus in this latter category has been to identify molecular signatures that can be used to predict the spread of HCC and to classify chronic liver disease patients who have the potential to develop HCC. NCI researchers have recently analyzed global gene expression patterns in HCC tumors and identified subclasses of HCC that are highly associated with patient survival. The biological differences identified in these subclasses are expected to provide attractive therapeutic targets for certain patients with HCC. NCI intramural investigators are also conducting translational research related to early drug development including the evaluation of novel treatments with immunotherapy or vaccines, radiation, and regional therapies as well as innovative molecular imaging methods for better staging and diagnosis of this cancer.

Thus, the NCI continues to be actively involved in support of research into the etiology and underlying molecular biology of liver cancer as well as research related to prevention, early detection, and treatment. Through its own divisions and its interactions with the other NIH ICs, the NCI has cosponsored ongoing clinical trials, research activities, and conferences that contribute significantly to our knowledge of liver cancer. These activities and future initiatives represent a robust research program that is poised to address the most urgent areas requiring additional investigation in this disease.

**Basic Lymphoma Research**

Lymphoma continues to be an area of specific interest in both the laboratory and the clinic, with increasing emphasis on translating preclinical data into clinical testing. NCI-funded research covers a broad spectrum of efforts.

The NCI extramural basic research portfolio contains a large number of research grants dealing with the molecular and cellular studies of lymphoma and its pathogenesis. Some recent advances in our understanding of lymphoma and its therapy are summarized below.

- A protein regulating the transcription of genetic information, called CBP, is a tumor suppressor. When this protein is deleted or inactivated in mouse thymoctyes it results in lymphoma. It appears that CBP regulated the levels of proteins that control the cell division cycle and that the appropriate regulation of these cell cycle proteins results in normal lymphocytes becoming lymphoma.

- CD20 is a B-lymphocyte specific protein that also regulates cell cycle progression during B-cell activation. The majority of B-cell malignancies express CD20. Thus, it has become an effective target for therapy of non-Hodgkin lymphoma. Recent work has developed new monoclonal antibodies against CD20 and a CD20 genetic knockout mouse to study CD20 function in detail *in vivo*. These new reagents should allow us to gain a better understanding of the molecular mechanisms that influence anti-CD20 immunotherapy.

- Recent work on the mechanism of action of anti-CD20 monoclonal antibodies against lymphoma has revealed that the complement system is not required for effective anti-CD20 immunotherapy. The B-cell depletion was dependent on an intact monocyte-macrophage network and their associated cell surface receptors. This insight might explain the failure of anti-CD20 therapy in myelosuppressed patients with reduced numbers of tissue monocytes. This information will be valuable in improving the effectiveness of anti-CD20 immunotherapy.

**RAID and RAND Lymphoma Studies to Begin**

The Rapid Access to Intervention Development (RAID) and Rapid Access to NCI Discovery Resources (RAND) programs were initiated to fulfill the recommendation of the Leukemia, Lymphoma, and Myeloma Progress Review Group to develop resources to rapidly translate lead structures into therapeutic agents. Numerous applications have been reviewed and approved. Compounds in development for the treatment of MDS, leukemia, lymphoma, and multiple

myeloma include monoclonal antibodies, radiolabeled or toxin-conjugated monoclonal antibodies, vaccines, oligodeoxynucleotides, cytokines, triterpenoids, 8-chloro-AMP, apogossypol, receptor-targeted-liposomal daunorubicin, imexon, subnanoparticles of selenium, and small molecules that target the BCR/ABL oncoprotein.

**Study Agents for Clinical Lymphoma Research**

New generations of established agents and first-generation molecularly targeted agents are now in clinical trials. These novel agents span a broad spectrum of mechanisms. They are evaluated both as single agents and in combination with other approved and other experimental agents. Agents in the clinic supported by NCI include the following:

*Approved Drugs*: Approved drugs under evaluation for new indications or novel combinations: Campath-1H antibody, rituximab, IDEC-Y2B8 (anti-CD 20 radioimmunoconjugate), bortezomib (Velcade - proteosome inhibitor), thalidomide, arsenic trioxide, oxaliplatin, gemcitabine.

*Experimental agents*: Hu1D10 antibody, LMB-2 antibody, triapine (small molecule ribnonucleotide reductase inhibitor), GTI-2040 (antisense ribonucleotide reductase oligonucleotide), flavopiridol (CDK inhibitor), MLN 518 (tyrosine kinase inhibitor), BMS-247550 (epothilone B analogue), CCI-779 (rapamycin analogue), UCN-01 (CDK inhibitor), bryostatin (CDK inhibitor), tipifarnib (FTI inhibitor), interleukin-12, 506U78 (AraG prodrug), depsipeptide, (histone deacetylase inhibitor), SAHA (histone deacetylase inhibitor), HeFi-1 (anti-CD30 antibody), G3139 (antisense Bcl-2 oligonucleotide).

A new regimen for flavopiridol, based upon pharmacokinetic data from earlier studies as well as new information on the protein binding of this drug, has shown striking activity in indolent leukemias and will now be evaluated in lymphomas.

Over 80 studies specific to patients with lymphoma are directly sponsored by the NCI, with more studies that include lymphoma along with other diseases.

**Recent Data from NCI-sponsored Lymphoma Research**

Mature data from multiple clinical studies seeking to define the best use of rituximab were released within the last year. Despite high overall response rates, indolent NHL is characterized by continuous relapse. A study performed by the Eastern Cooperative Oncology Group (ECOG), evaluated the ability of 2 years of maintenance rituximab to prolong remission. The trial showed that maintenance rituximab significantly prolongs progression-free survival (PFS) after CVP chemotherapy in patients with advanced indolent NHL. Extended follow-up will determine whether longer PFS translates into improved survival. Aggressive NHL is curable in approximately half of patients. The addition of rituximab to CHOP has improved cure rates in B-cell aggressive NHL. Another ECOG study confirmed the benefit of adding rituximab to chemotherapy for patients with these diseases and supported the use of maintenance therapy with rituximab in selected patients.

Data from multiple studies evaluating alternative forms of allogeneic hematopoietic stem cell transplantation have become available within the last year. Immunoablative approaches, where lower doses of chemotherapy still allow for an immune system transplant, consistently suggest less toxicity with significant graft versus lymphoma effects. Continued efforts at modifying the graft anti-tumor and anti-host effects will improve the safety of this approach, which may provide long-term disease control. To test the merits of these two approaches (auto transplant with rituximab and non-myeloablative allotransplant), the Blood and Marrow Transplant Clinical Trials Network, which is cofunded by NCI and NHLBI, has just launched a phase II/III multicenter study. Approximately 350 patients will be enrolled at more than 20 transplant centers in this 6-year study. Patients with a matched sibling donor will receive a nonmyeloablative allo transplant. Patients without a matched donor will receive an auto transplant followed by maintenance therapy with rituximab.

**Phase II Trial for Melanoma**

NCI recently funded a phase II melanoma clinical trial, targeting a specific enzyme defect—argininosuccinate synthesize deficiency found in melanoma cells. Malignant melanoma is usually resistant to drug therapy, which historically has been nonselective in action and often very toxic. This trial takes advantage of the fact that exposure of melanoma cells to arginine deiminase, an enzyme that catalyzes the hydrolysis of arginine to citrulline, results in melanoma cell arginine starvation and apoptotic cell death while minimizing toxicity. A total of 43 stage IV metastatic melanoma patients will be recruited and evaluated in this trial, and parallel molecular correlative assays will be conducted to elucidate the mechanisms of apoptotic cell death, drug resistance, and treatment effect.

**Ongoing and Future Research in Myelodysplasia and Myeloproliferative Disorders**

Ongoing clinical research into therapeutic interventions for MDS/MPD include evaluation of the promising drugs bortezomib (Velcade), tipifarnib (Zarnestra), bevacizumab, imatinib (Gleevec), flavopiridol, Campath-1H, bryostatin, MS-275, epithilone-B, triapine, CCI-779, thalidomide, revlimid, phenylbutyrate, and gemtuzumab ozogamycin. Additional studies are exploring immunoablative hematopoietic stem cell transplantation and leukemia vaccines.

The NHLBI released an RFA (HL-04-034) titled “Cellular and Genetic Discovery toward Curative Therapy in Myeloproliferative Disorders (MPD)” on May 21, 2004. The NCI is participating in this RFA by providing FY2005 funds to support up to 12 new grants in response to this RFA. The application receipt date is February 16, 2005.

**Rapamycin and Tuberous Sclerosis**

NCI is funding a nonrandomized open label pilot study using rapamycin to reduce or eliminate renal angiomyolipomas associated with either tuberous sclerosis or sporadic lymphangioleiomyomatosis through the Quick Trials for Novel Cancer Therapies initiative. The target accrual is 30 adult patients, with 23 patients already on the trial. This trial takes advantage of the recent discovery of a novel therapeutic target, mTOR kinase, which is involved in regulating

cell cycle control and cell growth. Modern imaging methods are being used to diagnose, monitor, and validate rapamycin's therapeutic effects.

As a follow up to this study, in December 2004, NCI will fund a consortium to conduct the first multicenter clinical trial for tuberous sclerosis—a phase II trial of rapamycin.

## B. Intramural Projects

### Molecular Signature Identified for Liver Cancer

A molecular signature has been identified that can be used to predict metastatic hepatocellular cancer (HCC) and patient prognosis. This signature can separate HCC patients into metastatic and nonmetastatic groups and may be useful for diagnosing HCC patients with metastatic potential. Currently, a cross-validation study is being conducted with a larger number of independent HCC patients to refine this signature. A serum profile has been established by determining the serum concentration of osteopontin and matrix metalloproteinase 9 to predict HCC patient survival and metastasis. Both microarray and serum profiling may provide a cross-validation of the metastasis prediction model in preparation for whether it should be recommended for aiding future clinical diagnosis.

### Clinical Trials in Pediatric Sarcoma and Leukemia Patients

The Pediatric Oncology Branch emphasizes clinical trials in high-risk sarcoma and leukemia patients and new drug development studies for pediatric cancer patients. Current efforts in high-risk sarcoma patients include development of effective immunotherapeutic strategies and utilization of allogeneic bone marrow transplant to generate graft-versus-tumor effects. Similar strategies are being used for recurrent leukemia patients. In addition, specific immunotoxin therapy for recurrent leukemias is currently being evaluated.

The value of dynamic MRI imaging in predicting response to neoadjuvant chemotherapy is being studied in newly diagnosed osteosarcoma. The predictive value appears to be promising. Preliminary analysis of gene expression profiling on these tumors suggests that profiles may cluster patients into "good" and "poor" prognosis prospectively.

IRB approval was recently received for a new salvage therapy for recurrent osteosarcoma and Ewing's sarcoma using a combination of sequential gemcitabine/docetaxel based on preliminary *in vitro* synergy and previous activity of the single agents. This study will be performed through a newly formed sarcoma consortium and is supported by Aventis and Lilly.

Current research efforts include clinical trials of novel, nonmyeloablative allogeneic stem cell transplant regimens for childhood hematopoietic malignancies. Biologic correlative studies conducted as part of these trials include valuation of chemotherapy-induced immunosuppression, immune recovery after allogeneic transplantation, and the pathophysiology of graft-versus-host disease. Additional clinical trial activities include phase I development of immunotoxins for refractory leukemias and lymphomas. In an effort to improve understanding of the molecular

mechanisms of leukemogenesis and identify potential new therapeutic targets, collaborative investigations of gene expression profiles of pediatric leukemias are also being conducted.

**Molecular Markers of Ewing's Sarcoma and Rhabdomyosarcoma Aid Diagnosis**

Many pediatric solid tumors exhibit fundamental cytogenetic abnormalities that have implications in their pathogenesis. The Ewing's sarcoma family of tumors (ESFT) and alveolar rhabdomyosarcoma (RMS) are characterized by consistent chromosomal translocations that result in the fusion of genes and subsequent formation of novel chimeric genes. These molecular markers can be detected by RT-PCR or fluorescence *in situ* hybridization (FISH) and can be used not only to establish the diagnosis in difficult cases but also to understand the pathogenesis of these tumors. Recently, the products of these fusion genes have become the target of vaccine therapies in newly established protocols in the Center for Cancer Research at the NCI.

**Novel Therapies Devised for Brain and Spinal Cord Tumors**

Novel experimental therapeutics are being developed for children and adults with tumors of the brain and spinal cord. Toward this end, translational laboratory efforts are focusing on new strategies for selective tumor targeting through gene transfer using neural and endothelial stem cells and novel genetic vectors, through the identification of tumor-selective processes such as angiogenesis, and through the identification of tumor-specific markers.

Angiogenesis inhibition is being explored as a novel therapeutic strategy for the treatment of malignant gliomas. Selective delivery of genes of choice to tumor-associated angiogenesis can result in subsequent destruction of the tumor vascular bed. This novel strategy will be brought to the clinic soon.

Scientists are additionally in the process of identifying unique proteins expressed on infiltrating glioma cells and on endothelium, associated with the angiogenic response of tumors, for the purpose of identifying novel peptides and single chain antibody molecules. These molecules will be tagged with radioisotopes for diagnostic purposes and conjugated with immunotoxins.

**Gene Expression Profiling Predicts Outcome in Rhabdomyosarcoma and Neuroblastoma**

NCI research scientists have developed substantial efforts in both proteomic and genomic characterization of pediatric tumors. Researchers have carried out reverse-phase protein arrays on set of 34 rhabdomyosarcomas. Expression of several proteins was highly associated with a high risk for relapse. Gene expression profiling is being performed on a series of neuroblastomas of different stages and prognosis to identify tumor-specific expression patterns or "fingerprints." The analysis has identified patterns of gene expression that accurately predicted outcome. These studies are also continuing in an attempt to identify new potential targets for therapy in poor outcome patients.

## Microarray for Neurofibromatosis Developed to Identify Molecular Targets

NCI scientists are developing a tissue microarray for pediatric solid tumors and neurofibromatosis type 1 (NF1)-associated tumors with the goal of analyzing these tumors for the presence of targets of new molecularly targeted agents. The microarray will be used to make rational decisions in the drug development for pediatric cancers and NF1.

## Molecular Signature of Malignant Gliomas Sought

Researchers are in the process of constructing a glioma-specific cDNA microarray chip to develop a molecular classification scheme of malignant gliomas. Through the identification of signature gene expression profiles, they hope to be able to group pediatric and adult gliomas into tumors that are biologically more related. This will allow us to offer more accurate prognoses and begin to address the issue of individualized therapies.

## Metastasis and Therapy Studied in New Mouse Models of Metastatic Osteosarcoma

Pediatric cancer researchers at NCI have a major focus on osteosarcoma. Researchers have recently developed mouse models of metastatic osteosarcoma and identified novel proteins that appear to play a major role in metastatic behavior in this model and, hopefully, in human osteosarcoma as well. Work is ongoing to determine what signaling pathways mediate these effects on metastatic behavior with the hope that novel therapeutic interventions can be identified and tested for patients with metastatic osteosarcoma. They are also testing novel anti-metastatic agents using this mouse model.

## Artificial Neural Networks Predict Clinical Outcomes in Neuroblastoma

Researchers at the NCI have used artificial neural networks (ANNs) and DNA microarrays to successfully predict the clinical outcome of patients diagnosed with neuroblastoma (NB). The ANNs also identified a minimal set of 19 genes whose expression levels were closely associated with this clinical outcome.

Using the 19 predictor genes, the ANNs were also able to partition the subset of patients classified as high risk into good and poor outcome groups as well as predict which of the high-risk patients would fail conventional therapy. This has major clinical implications since we are now able to distinguish a group of ultra-high-risk patients who will not respond to conventional therapy and therefore require alternative treatment strategies. It may also be possible to reduce the intensity and thereby reduce the toxicity of the treatment regime to those predicted to survive based on their gene expression profile.

NCI scientists can translate findings to the clinic because simple prognostic assays can be developed based on this small number of genes. In fact, three of the genes found to be over-expressed in poor outcome tumors encode proteins secreted into the blood, meaning they could be used as serum prognosis markers in a simple blood test. In collaboration with industry, NCI researchers are now developing clinical assays based on these 19 genes and planning to test for the presence of these serum markers in other patients with NB for the prognostic prediction.

## Targeted Treatment Agents for Neurofibromatosis Tested in Clinical Trials

The ras family of G-proteins play an important role in the transduction of signals that trigger cell proliferation, and mutations in ras genes are found in 30 percent of all human cancers. Ras proteins undergo post-translational farnesylation, which is required for activity of wild-type and mutant ras proteins, and this step can be inhibited by farnesyltransferase inhibitors, such as R115777. The evaluation of R115777 in children with refractory solid tumors and NF1 is therefore a rational choice. A phase I trial of R115777 for children with these tumors was recently completed, and based on the results of this phase I trial, a multi-institutional, randomized, double-blinded, placebo-controlled, cross-over phase II trial of R115777 for patients with NF1 and progressive plexiform neurofibromas was developed and is open for patient accrual.

A phase I trial of the antifibrotic agent pirfenidone is coordinated by the NCI researchers in collaboration with Children's National Medical Center, Washington, DC, and the Mayo Clinic, Rochester, MN, and just completed accrual. Subsequently a phase II trial of pirfenidone for children and young adults with NF1 and progressive plexiform neurofibromas was developed at the NCI, and this trial just opened for accrual.

## Gene Expression Profiling Diagnoses Benign versus Malignant Thyroid Tumors

Classification of human cancers into distinct groups based on their molecular profile rather than their histological appearance may prove to be more relevant to specific cancer diagnoses and cancer treatment regimes. Therefore, in this study, the NCI developed a gene expression approach to diagnose benign versus malignant thyroid lesions in 73 patients with thyroid tumors. Researchers successfully built a 10- and 6-gene model able to differentiate benign versus malignant thyroid tumors. Their results support the premise that a molecular classification system for thyroid tumors is possible, and this in turn may provide a more accurate diagnostic tool for the clinician managing patients with suspicious thyroid lesions.

## A Functional Polymorphism in RGS6 Modulates Bladder Cancer Risk

RGS proteins negatively regulate G protein signaling. Recent reports have shown that RGS proteins modulate neuronal, cardiovascular, and lymphocytic activity, yet their role in carcinogenesis has not been explored. In an epidemiologic study of 477 bladder cancer patients and 446 matched controls, three noncoding single-nucleotide polymorphisms (SNPs) in RGS2 and RGS6 were each associated with a statistically significant reduction in bladder cancer risk. The risk of bladder cancer was reduced by 74 percent in those individuals with the variant genotype at all three SNPs. In addition, the NCI demonstrated that RGS2 transcripts and several splice variants of RGS6 are expressed in bladder cancer cells. These data provide the first evidence that RGS proteins may be important modulators of cancer risk and validate RGS6 as a target for further study.

In an ongoing hospital-based case-control study, NCI researchers explored the association between 12 noncoding SNPs in 5 RGS genes identified in the National Center for Biotechnology Information (NCBI) database (dbSNP) and the risk of bladder carcinoma, a cause of over 12,000 deaths per annum in the United States. Results suggest that selected RGS variant SNPs may be

important modifiers of cancer risk. To validate the biological significance of these SNPs, researchers also sought to identify functional changes in transcript levels, alternative splicing events, and protein translation efficiency that may result from the presence of the variant alleles.

**Novel RNA-based Antiviral Therapies Devised for Cervical Cancer**

The papillomaviruses are epitheliotropic small DNA tumor viruses that cause both benign and malignant lesions in humans and animals. An oncogenic subset of the human papillomaviruses (including HPV-16, -18, -31, and -33) is associated with the vast majority of cervical cancers as well as squamous cell carcinomas in other locations. Expression of papillomavirus genes is regulated at both transcriptional and posttranscriptional levels. NCI researchers are currently exploiting HPV alternative splicing to develop novel RNA-based antiviral and anticancer therapies. In addition they have been involved in several collaborations on HPV transcriptional and posttranscriptional regulation. Some of the techniques they have developed, such as isoform-specific real-time quantitative RT-PCR (QRT-PCR), are also being used in other collaborations both within and outside the papillomavirus field.

Targeting HPV-infected cells using specific trans-splicing: The ideal cancer therapy would kill cancer cells and have no effect on normal cells. The expression of spliced human papillomavirus E6/E7 pre-mRNAs by the vast majority of cervical cancers provides an absolute difference between cancer cells and normal cells that can be exploited for the specific targeting of these cells. Through a cooperative research and development agreement (CRADA) between NCI and Intronn, Inc., researchers are investigating the use of Intronn's Spliceosome Mediated RNA Trans-splicing (SMaRT) Technology to develop a novel RNA-based suicide gene therapy for cervical cancer.

**Vaccines Developed Against HPV Virion Proteins**

There is a strong association between malignant progression of human genital lesions and certain human papillomavirus (HPV) types, most frequently HPV-16. NCI research is concerned with development of vaccines against HPV and other targets and elucidation of the PV life cycle. NCI research scientists have generated virus-like particles (VLPs) for HPV-16 and other PVs that consist of the L1 major capsid protein or L1 plus L2, the minor capsid protein. Parenteral injection of purified VLPs induced high titers of neutralizing antibodies and protection from experimental challenge in animal models. Based upon these results, they have validated GMP-grade VLPs and have completed phase 1 and phase 2 clinical trials of an HPV16 L1 VLP vaccine. Vaccines, even those vaccinated in the absence of adjuvant, consistently produced high titers of HPV-16 psuedovirion neutralizing antibodies and reported only minor side effects. Researchers are also developing alternative vaccine candidates, some for use in disease induced by HPV and others for use in diseases unlinked to HPV infection.

**Molecular Genetics of Gynecologic Cancers Characterized**

Gynecologic cancer remains a major health problem for women in this country with approximately 25,000 deaths annually attributed to this problem. The purpose of this project is to characterize the molecular genetics of this group of tumors and ultimately use that information for clinical application in rationally designing therapeutic and prevention trials. NCI scientists have

characterized endometrial, cervical, and ovarian specimens that span the histiologic spectrum from benign to malignant for mutations in the ras, p53, cyclin dependent kinase inhibitors, FHIT and Rb genes, and microsatellite instability. These studies should help to identify the molecular genetic events that are important in the genesis of endometrial and cervical cancers and their use for their early detection.

Evaluation of ovarian tumors revealed that activated ras genes are found in benign (10 percent) and low malignant potential (LMP) tumors (30 percent) but not ovarian carcinomas (5–10 percent). In addition, mutations in the tumor suppressor genes p53 and Rb occur in ovarian carcinomas (48 and 14 percent respectively) but are not present in LMP tumors. This suggests that ovarian carcinoma and LMP tumors are discrete biologic entities.

To identify potential new markers of ovarian cancer and genes important in its pathogenesis, malignant ovarian epithelium is being compared to its benign counterpart using differential display technology, representational display, and microarrays. As part of the Director's Challenge collaborative grant between this laboratory and MSK, 400 ovarian cancer specimens will be profiled utilizing cDNA microarrays and patterns will be correlated with clinical characteristics such as survival, histology, and stage. Genes that are differentially expressed will be isolated, cloned, and characterized for their role in the development of ovarian cancer.

**Genome Expression Profiling of Ovarian Cancer Reveals Activated Pathways**

Ovarian cancer is the most lethal type of gynecologic cancer in the Western world. The high case fatality rate is due in part because most ovarian cancer patients present with advanced stage disease that is essentially incurable. In order to obtain a whole genome assessment of aberrant gene expression in advanced ovarian cancer, NCI research scientists used oligonucleotide microarrays comprising over 40,000 features to profile 37 advanced stage papillary serous primary carcinomas. We identified 1191 genes that were significantly ($P < 0.001$) differentially regulated between the ovarian cancer specimens and normal ovarian surface epithelium. The list of differentially expressed genes includes ones that are involved in cell growth, differentiation, adhesion, apoptosis, and migration. Based on our expression results, a signaling pathway associated with tumor cell migration, spread, and invasion was identified as being activated in advanced ovarian cancer. The data generated in this study represent a comprehensive list of genes aberrantly expressed in serous papillary ovarian adenocarcinoma and may be useful for the identification of potentially new and novel markers and therapeutic targets for ovarian cancer.

**Recombinant Immunotoxins Developed for Ovarian and Pancreatic Cancer Therapy**

Mesothelin is a differentiation antigen present on normal mesothelial cells and overexpressed in several human tumors, including mesothelioma and ovarian and pancreatic adenocarcinoma. Mesothelin is a promising candidate for tumor-specific therapy, given its limited expression in normal tissues and high expression in several cancers. We have developed a recombinant anti-mesothelin immunotoxin (SS1P) that targets ovarian cancers, mesotheliomas, and pancreatic cancers and is currently in clinical trials. Recombinant immunotoxin SS1P shows significant activity in preclinical models including complete regression of mesothelin-expressing tumor xenografts in mice as well as cytotoxic activity *in vitro* against tumor cells obtained directly from

patients with ovarian cancer and peritoneal mesothelioma. NCI researchers are using animal models to evaluate therapies that could enhance antibody delivery, with the goal of translating these findings to the clinic. Two phase 1 trials with immunotoxin SS1P are now open.

**Targeted Systemic Radiotherapeutics Evaluated for Intraperitoneal Cancers**

Evaluations are being performed of the therapeutic efficacy of targeted systemic radiotherapeutics for treating metastatic intraperitoneal cancer, such as pancreatic cancer, while simultaneously optimizing their value in conjunction with chemotherapeutics. The majority of targeted ratation therapies have employed a single administration of a single isotope, while in fact, dose fractionation and combination therapies with other modalities clearly provide superior results. Studies combining the use of targeted α-emitters with chemotherapeutics are novel and unique to the NCI.

**Cytogenetic Signature of Vaginal Squamous Cell Carcinoma Identified**

NCI intramural scientists used comparative genomic hybridization to establish a pattern of genomic imbalances in vaginal squamous cell carcinomas. Analysis of 16 tumors revealed that 70 percent of vaginal carcinomas carry relative copy number increases that map to chromosome arm 3q. Other recurring gains were observed on chromosome arms 5p and 19p. Chromosomal losses were infrequent. The cytogenetic data were related to the presence of human papillomavirus genomes, expression of laminin-5 as a marker for invasiveness, and expression levels of markers for proliferative activity and mutated TP53. The results suggest that vaginal carcinomas are defined by a specific distribution of chromosomal aneuploidies and that the pattern of genomic imbalances is strikingly similar to that observed in squamous cell carcinomas of the uterine cervix. Age at diagnosis, tumor size, and increased laminin-5 expression have a significant influence on the survival time. As in other malignant diseases, early detection greatly affects survival rates. It is therefore a perceived goal to analyze and understand the genetic mechanisms leading to vaginal tumorigenesis.

**Techniques Evaluated for Early Detection of Esophageal Cancer**

Esophageal cancer has a very poor prognosis, primarily because most tumors produce symptoms only after they have spread beyond the esophageal wall and are unresectable. Significant improvement in survival will require successful strategies to diagnose and treat more cases at earlier, more curable stages of the disease. A project is being carried out in Linxian, China, a county with very high rates of esophageal cancer, to evaluate techniques that may be useful in a practical early detection and treatment program. The project includes five studies: the Cytology Sampling Study, the Mucosal Staining Study, the Endoscopic Staging Study, the Endoscopic Therapy Pilot Study, and the Chemoregression Study.

**Molecular Genetics of Kidney Cancer Studied**

In order to identify the genes that cause kidney cancer and develop molecular therapeutics for this disease, we have studied the inherited forms of cancer of the kidney. Individuals with the inherited form of kidney cancer associated with von Hippel Lindau (VHL) are predisposed to develop

tumors in the brain, spine, eyes, pancreas, adrenal gland, and inner ear. By studying VHL families we were able to perform genetic linkage analysis to localize and subsequently identify the VHL gene on chromosome 3. NCI scientists have identified the VHL gene mutation in the germline of 246/246 VHL kindreds and are currently studying genotype/phenotype relationships as well as evaluating minimally invasive forms of therapy for kidney and adrenal tumors in VHL. The VHL gene has been shown to be the gene for the hereditary form of renal carcinoma associated VHL as well as the common form of sporadic (nonhereditary) kidney cancer (clear cell renal carcinoma).

**Angiogenesis in Renal Cell Cancer Targeted in Laboratory and Clinical Studies**

Renal cell cancer (RCC) is a highly vascular tumor that contains a mutation in the VHL tumor suppressor gene in over 80 percent of clear cell carcinomas. This mutation is linked to deregulation of vascular endothelial growth factor (VEGF), a potent angiogenic agent. NCI researchers are currently beginning a new initiative directed at identifying the mediators of angiogenesis and growth of RCC and neutralizing these biological activities in preclinical tumor models and in patients with RCC. In preclinical models of RCC xenografts in nude mice, we are evaluating neutralizing antibodies to FGF-5. RCC was found to express FGF-5 when expression screening of a cDNA library from an RCC line revealed FGF-5 as the targeted RCC-associated tumor antigen. This molecule, originally identified by its ability to transform 3T3, is expressed in approximately 60 percent of RCC lines and some prostate and breast cancer lines. Researchers have not found it expressed by normal adult tissues by RT-PCR, and it therefore represents an excellent therapeutic target for multiple adenocarcinomas including RCC.

Parallel to these laboratory studies, the NCI is conducting clinical protocols of anti-angiogenic agents. In a new, accruing clinical protocol, we are evaluating the efficacy of a humanized neutralizing antibody to hVEGF (Genentech, Inc.) in a randomized, double-blind, placebo-controlled format in patients with progressive, metastatic RCC. In this 150-patient trial, we are also evaluating the use of novel noninvasive imaging techniques to evaluate anti-angiogenic agents in clinical trials. The next generation of clinical studies will be combinations of agents if the anti-VEGF monoclonal antibody demonstrates any significant clinical activity.

**Gene Expression Profiles Provide Diagnosis and Prognosis of Lymphoid Malignancies**

To provide a molecular basis for the diagnosis of human lymphoid malignancies, NCI researchers are exploiting DNA microarray technology to profile gene expression in these cancers on a genomic scale. An NCI laboratory created a novel DNA microarray, the "Lymphochip," which is enriched in genes that are expressed in and/or function in lymphocytes. They have used Lymphochip and Affymetrix microarrays to profile gene expression in diffuse large B-cell lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma, multiple myeloma, and in a wide variety of normal lymphoid subsets.

One central goal of these studies is to relate gene expression to clinical outcome, thereby establishing a quantitative, reproducible, and informative molecular diagnosis of the lymphoid malignancies. The studies have revealed previously unknown types of diffuse large B-cell lymphoma that are indistinguishable by current diagnostic methods but have strikingly distinct gene expression profiles, originate from different stages of B-cell differentiation, utilize distinct

oncogenic mechanisms, and differ in their ability to be cured by current chemotherapy. For several lymphoid malignancies, we have identified molecular profiles that predict the length of survival or the ability to be cured by chemotherapy, thereby providing clinically useful prognostic indicators. A major effort has been mounted to create a diagnostic microarray that could provide these molecular diagnoses and prognoses to patients with lymphoid malignancies.

Importantly, the genes that are associated with clinical prognosis have provided new targets for therapy of the lymphoid malignancies. Functional genomics, chemical genetics, and molecular biological techniques are being used to validate these and other molecular targets toward the ultimate goal of targeted therapies for patients aimed directly at the disordered regulatory biology of their individual tumors.

**IGF-I Induces Migration and Invasion of Multiple Myeloma Cells**

Multiple myeloma (MM) is an incurable form of cancer characterized by accumulation of malignant plasma cells in the bone marrow. During the course of this disease, tumor cells cross endothelial barriers and home to the bone marrow. In later stages, myeloma cells extravasate through blood vessels and may seed a variety of organs. Insulin-like growth factor I (IGF-I) is one of several growth factors shown to promote the growth of MM cells. In this study, NCI researchers have assessed the ability of IGF-I to serve additionally as a chemotactic factor affecting the mobility and invasive properties of these cells. Results indicate that IGF-I promotes transmigration through vascular endothelial cells and bone marrow stromal cell lines. The identification of IGF-I as both a proliferative and migratory factor provides a rational basis for the development of targeted therapeutic strategies directed at IGF-I in the treatment of MM.

**Novel Therapeutic Strategies for Lymphoma Assessed in Clinical Trials**

*In vitro,* overexpression of the mdr-1 gene product, P-glycoprotein (Pgp), in tumor cells can confer high-level resistance to natural product-derived cytotoxics. It has been reported that Pgp was detectable by immunohistochemistry in 1/49 (2 percent) of untreated but was detectable in 6/8 (75 percent) of treated lymphomas, suggesting that Pgp conferred drug resistance. To test this hypothesis, NCI scientists developed and tested an mdr-1 reversal strategy in relapsed lymphomas using EPOCH (doxorubicin/vincristine/etoposide, prednisone, cyclophosphamide) and dexverapamil. Based on results showing EPOCH to be effective and well tolerated, they began a phase II study of EPOCH in previously untreated patients with aggressive lymphomas. In this study, EPOCH doses are escalated within patients to the maximum tolerated dose (MTD). End points are dose-intensity, efficacy, toxicity, and molecular markers of drug resistance. Early results show a high complete response rate of 89 percent with an EFS of 77 percent at 2 years median follow-up. Accrual to this trial continues.

NCI staff have recently developed and tested a "second-generation" EPOCH regimen (EPOCH II) to replace stem cell transplant for lymphomas requiring high-dose intensity including poor-prognosis untreated aggressive lymphomas, potentially curable relapsed lymphomas, and low-grade lymphomas. This regimen is based on experimental/clinical observations that suggest infusion schedules may improve the therapeutic index of natural product-derived cytotoxics and

that high-dose alkylator therapy can overcome drug resistance in lymphoma. Preliminary results show EPOCH II to have a response rate of 90 percent with 66 percent complete.

Examination of tumor tissues to assess potential mechanisms of drug resistance is an ongoing effort. Researchers have demonstrated the association of clinical drug resistance with p53 mutation and low proliferation rate in relapsed lymphomas. Currently, they are studying other novel cell cycle proteins such as p27 in lymphomas. Recently, we have been interested in testing novel protein kinase inhibitors in lymphomas. One such inhibitor, UCN-01, has shown marked synergy with fludarabine in a variety of human cell lines. To further assess this drug, the NCI will soon begin a phase I study of UCN-01 and fludarabine in patients with relapsed and refractory indolent lymphomas.

**Gynecologic Surgery Reduces Risk of Ovarian Cancer in BRCA Mutation Carriers**

Women with BRCA1 and BRCA2 mutations are reported to have a low risk of peritoneal carcinoma in the first years after bilateral oophorectomy (BO). To assess the level and persistence of reduction of ovarian and peritoneal cancer risk after gynecologic surgeries, 847 Israeli women with incident ovarian cancer or primary peritoneal cancer were tested for the three Ashkenazi founder mutations. A comparison of gynecologic surgery history among all case patients, BRCA1 (n = 187) and BRCA2 (n = 64) carrier case patients, and the noncarrier case patients (n = 598) to control subjects drawn from a population registry (n = 2,396) found that 8 women with primary peritoneal cancer and 128 control subjects reported a previous BO. Other gynecologic surgeries were associated with a 30–50-percent reduced risk of ovarian cancer, with the removal of some ovarian tissue associated with the most risk reduction. Reduced risks were seen in BRCA1/2 carriers and in noncarriers. Age at surgery and years since surgery did not affect risk reductions, but type and extent of surgery did. *J Natl Cancer Inst* 2003; 95:1072–1078.

**Ovarian Cancer Risk after Use of Ovulation-Stimulating Drugs**

A retrospective cohort study of 12,193 eligible study subjects (median age 30 years), who were evaluated for infertility during the period of 1965 to 1988 at 5 clinical sites, identified 45 subsequent ovarian cancers. The infertility patients had a significantly elevated ovarian cancer risk compared with the general population (standardized incidence ratio = 1.98).When patient characteristics were taken into account and risks assessed in the infertile women, the rate ratios (RR) associated with ever use were 0.82 for clomiphene and 1.09 for gonadotropins. There were higher but nonsignificant risks with follow-up time. Although drug effects did not vary by causes of infertility, a slightly higher risk was associated with clomiphene use among women who remained nulligravid, based on six exposed patients (RR = 1.75). These results do not confirm the strong link reported in some studies between ovulation-stimulating drugs and ovarian cancer. Slight but nonsignificant elevations in risk associated with drug use among certain subgroups of users, however, support the need for continued monitoring of long-term risks. *Obstet Gynecol* 2004; 103:1194–1203

**Tamoxifen and Risk of Rare Endometrial Cancers**

Recent studies suggest that the tamoxifen-related risk of uterine corpus cancer may be especially high for some uncommon cell types, although the magnitude of risk has not been quantified. Data from 39,451 breast cancer patients initially treated with tamoxifen were evaluated. The overall risk of subsequent uterine corpus cancer was increased more than 2-fold (observed-to-expected ratio [O/E] = 2.17) relative to the general SEER population. Relative risk was substantially higher for malignant mixed mullerian tumors (MMMTs) (O/E = 4.62) than for endometrial adenocarcinomas (O/E = 2.07), although excess absolute risk was another 1.4 versus 8.4 cancers per 10,000 women per year, respectively. Among women who survived for 5 years or longer, there was an 8-fold relative risk for MMMTs and a 2.3-fold risk for endometrial adenocarcinomas, with those developing MMMTs having a worse prognosis. These findings indicate that tamoxifen may have delayed effects, such as the increased risk of MMMTs, rare but aggressive tumors of unclear pathogenesis. *J Natl Cancer Inst* 2004; 96:70–74.

**Fumarate Hydratase Gene Mutations Cause Hereditary Leiomyomatosis and Renal Cell Cancer**

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal-dominant disorder characterized by smooth-muscle tumors of the skin and uterus and/or renal cancer. FH gene sequence analysis among 35 North American families with cutaneous leiomyomas revealed germline mutations in 31 families (89 percent). Twenty different mutations in FH were identified, of which 18 were novel. Of these 20 mutations, 2 were insertions, 5 were small deletions that caused frameshifts leading to premature truncation of the protein, and 13 were missense mutations. Eleven unrelated families shared a common mutation: R190H. Ninety-eight percent (46/47) of women with cutaneous leiomyomas also had uterine leiomyomas, and 89 percent (41/46) had a prior total hysterectomy, 44 percent at age = 30 years. Unilateral and solitary renal tumors were identified in 13 individuals from 5 families. Seven individuals from four families had papillary type II renal cell carcinoma, and another individual from one of these families had collecting duct carcinoma of the kidney. HLRCC is associated with FH mutations and clinically significant uterine fibroids and aggressive renal tumors. *Am J Hum Genet* 2003; 73: 95–106.

**Hepatitis C Virus Infection and Non-Hodgkin Lymphoma**

Several studies have noted elevated hepatitis C virus (HCV) prevalence among patients with non-Hodgkin lymphoma (NHL), suggesting that HCV infection increases NHL risk through chronic immune stimulation. In a population-based case-control study of NHL in the United States, HCV infection was identified using an enzyme immunoassay and confirmed by recombinant immunoblot assay or HCV RNA detection. The association between HCV and NHL was assessed using logistic regression, adjusting for demographic factors, illicit drug use, or medical history. Thirty-two of 813 (3.9 percent) NHL cases and 14 of 684 (2.1 percent) controls were HCV infected. Positive associations were noted for follicular, marginal zone and mucosa-associated lymphoid tissue NHLs. The study demonstrates an association between HCV infection and NHL in the United States and suggests that HCV infection may be a cause of NHL. *Int. J. Cancer* 2004; 111, 76–80.

**Obesity-related Cancer Increases among Black and White Men**

Obesity has been linked to excess risk for many cancers, but the evidence remains tenuous for some uncommon types and few studies have included nonwhite subjects. The risk for all cancer sites and subsites was examined among a cohort of male U.S. veterans (3,668,486 whites; 832,214 blacks) hospitalized with a diagnosis of obesity. Among white veterans, risk was significantly elevated for several cancers, including cancers of the lower esophagus, gastric cardia, small intestine, colon, rectum, gallbladder and ampulla of vater, male breast, prostate, bladder, thyroid, and connective tissue and for malignant melanoma, multiple myeloma, chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML). Excess risks initially observed for cancers of the liver and pancreas persisted among men without a history of diabetes or alcoholism. Among black veterans, risks were significantly elevated for cancers of the colon, extrahepatic bile ducts, prostate, and thyroid and for malignant melanoma, multiple myeloma, CLL, and AML. *Cancer Causes Control* 2004; 15:35–43.

**Risk of Head and Neck Squamous Cell Cancer and Death in Transplanted and Untransplanted Patients with Fanconi Anemia**

Hematopoietic stem cell transplant (SCT) is currently the only therapy that can restore normal hematopoiesis in patients with Fanconi anemia (FA). FA patients have a high baseline risk of squamous cell cancers (SCC) of the head, neck, and esophagus, and SCT conditioning may increase SCC incidence. The risks of SCC and death in 145 untransplanted FA patients in the North American Survey (NAS) cohort were compared to 117 transplanted FA patients in a separate cohort. The age-specific hazard of SCC was 4.4-fold higher in transplanted versus untransplanted FA patients, and SCC occurred at significantly younger ages in the former. Survival after SCC was similarly poor in both cohorts. The hazard of SCC increased at a greater than linear rate by 10 years after transplant. Acute and chronic graft versus host diseases were significant SCC risk factors. Adverse event rates in these cohorts provide historical control rates to assess emerging therapies for FA. *Blood First Edition Paper*, prepublished online August 26, 2004; DOI 10.1182/blood-2004-04-1652

**Alachlor Exposure May Increase Risk of Lymphohematopoietic Cancers**

Cancer incidence during 1993 to 2000 was evaluated among pesticide applicators exposed to alachlor in the Agricultural Health Study, a prospective cohort study of licensed pesticide applicators in Iowa and North Carolina. A total of 49,980 pesticide applicators were included in this analysis, including 26,510 applicators (53 percent) who reported alachlor use. Among alachlor-exposed applicators, a significant increasing trend for incidence of all lymphohematopoietic cancers was associated with lifetime exposure-days and intensity-weighted exposure-days to alachlor. The risks of leukemia (rate ratio [RR] = 2.8) and multiple myeloma (RR = 5.7) were increased among applicators in the highest alachlor-exposure category. *Am J Epidemiol* 2004; 159:373–80

**Formaldehyde Exposure May Increase Leukemia Risk**

Extended follow-up of a cohort of 25,619 U.S. industrial workers evaluated the association between measures of formaldehyde exposure (peak exposure, average exposure intensity, cumulative exposure, and exposure duration) and mortality from lymphohematopoietic cancers (n = 178). Compared with workers exposed to low peak levels of formaldehyde (0.1–1.9 ppm), workers exposed to peak levels of 2.0–3.9 ppm were two and a half times (RR= 2.43) more likely to develop myeloid leukemia. Workers exposed to peak levels of 4.0 ppm were three and a half times more likely (RR = 3.46) to develop myeloid leukemia. Compared with workers exposed to low levels of average exposure intensity of formaldehyde (0.1–0.4 ppm), relative risk for myeloid leukemia was slightly elevated (RR = 1.15) for workers exposed to 0.5–0.9 ppm and two and a half times more prevalent for workers exposed to 1.0 ppm average intensity (RR = 2.49). *J Natl Cancer Inst* 2003; 95:1615–1623

**Significant Ongoing Rare Diseases Research Initiatives**

**Cohort Consortium**

NCI has launched the Cohort Consortium, in which parallel and pooled studies of large population cohorts utilize advances in genomic technology to identify inherited susceptibility genes and gene-environment interactions in nonfamilial cancers. The Consortium is a unique public-private partnership that currently includes 23 population cohorts; epidemiologists for each cohort are collaborating not only with genomicists at their own institutions but also are working formally with three major genome centers: the Whitehead/MIT Center for Genome Research, the Centre d'Etude du Polymorphisme Humain (CEPH) in Paris, and the NCI Core Genotyping Facility.

The Cohort Consortium represents a coordinated, interdisciplinary approach that will greatly accelerate the research process and allow scientists to perform subset analyses and confirmatory studies to examine gene-environment and gene-gene interactions. The approach provides "instantaneous parallel replication" of findings across cohorts. The Cohort Consortium presents the cancer research community with an extraordinary opportunity to advance research on genes and the environment and to do so economically by using already existing resources. The unique epidemiologic infrastructure also provides an opportunity to partner with other NIH Institutes to investigate a series of complex diseases, including diabetes and cardiovascular and neurological diseases. By involving both the intramural and extramural research communities, the opportunity exists for NIH to cost effectively leverage its resources and ensure that the dramatic advances in genomics and other emerging technologies are incorporated into rigorous population-based studies to unravel the determinants and mechanisms underlying cancer and other diseases.

**Non-Hodgkin Lymphoma Case-control Consortium**

NCI intramural and extramural investigators have joined forces in a coordinated series of ongoing case-control studies focused on non-Hodgkin lymphoma (NHL). The NHL collaboration, known as InterLymph, represents a new generation of large-scale molecular epidemiology, with investigators pooling data from North America, Europe, and Australia to identify reasons for the increasing incidence of this tumor around the world. Each case-control study includes a detailed

review of the pathological and genetic characteristics of the NHL cases. The investigators are sharing data in order to test for genetic and environmental causes that cannot be addressed in individual studies with smaller sample sizes. Because the consortium involves essentially all major ongoing epidemiologic studies of NHL, it represents a model for the study of many malignancies.

The first breakthrough finding from the consortium was the demonstration that the TNF-alpha gene plays a key role in diffuse large B-cell lymphoma, the most common form of the disease. A recent meta-analysis of 13 single nucleotide polymorphisms (SNPs) in genes that play a role in regulating the immune system revealed a statistically significant association between a gene variant of TNF-alpha and increased risk of developing diffuse large B-cell lymphoma. Individuals heterozygous for this SNP were at 30-percent increased risk, while homozygous individuals were at 60-percent increased risk of developing the disease. Other genes are now under investigation.

**Human Papilloma Virus Vaccine Trial**

A wealth of scientific evidence has led to the conclusion that virtually all cases of cervical cancer are attributable to cervical infection by a subset of human papilloma viruses (HPV). About one-half of cervical cancer is attributable to HPV-16 infection. The second most frequent type, HPV-18, accounts for another 10–20percent of these cancers. HPV infection, predominantly HPV-16, also appears to cause most anal cancers as well as a proportion of vulvar, penile, and head-and-neck cancers. An effective HPV vaccine should be able to reduce the incidence of the cancers attributable to HPV infection.

Investigators at the National Cancer Institute, with long-term support from the Office of Research on Women's Health (ORWH), have developed a method for producing an HPV vaccine composed of a single noninfectious protein from the virus. The scientists found: (1) multiple copies of the viral protein spontaneously assemble into empty shells (called virus-like particles or VLP) to which the immune system responds as though encountering the authentic virus; (2) the VLP vaccine is highly protective in animal papilloma virus models; and (3) an HPV-16 VLP vaccine is safe and induces a strong, durable immune response in women and men. Two pharmaceutical companies (Merck and GlaxoSmithKline) are currently carrying out international phase III vaccine trials, having found that the VLP vaccine can protect women from infection by the HPVs targeted by the vaccine. The NCI, with continued support from ORWH, is conducting an independent trial of the vaccine produced by GSK. The trial is being carried out in Costa Rica, where cervical cancer is the most common cancer of women, in close collaboration with Costa Rican investigators and the Government of Costa Rica. The experimental HPV vaccine, or a hepatitis A vaccine, is given in a blinded manner to all healthy young women who ask to participate. The women are followed for a period of 4 years for the development of HPV infection and cervical lesions, which are treated according to accepted standard of care guidelines. Thus, all women in the trial benefit from excellent cervical cancer screening, the control group has the benefit of receiving a vaccine that will protect them against hepatitis A, and the experimental group may benefit from the experimental vaccine. Cross-over vaccination and vaccination against hepatitis B will also be offered to all participants at the end of the trial, as an added benefit.

The prevention of cervical cancer is the main public health goal of the vaccine, which may become available to the public in 3–5 years, if the ongoing trials when completed are deemed successful by

the FDA. The current vaccines target HPV-16 and -18, which together account for about 60–70 percent of cervical cancer worldwide. If a vaccine is 90-percent effective against these HPV types, it will have the potential of reducing the incidence of cervical cancer by more than 60 percent. An effect of this magnitude would translate to a reduction of about 150,000 deaths per year worldwide from cervical cancer. In addition, the vaccine would be expected to reduce by about one-half the number of precancerous cervical lesions that require treatment (currently about 1 million cases per year in the United States), leading to a substantial reduction in morbidity, anxiety, and cost. In theory, the vaccine could also reduce the incidence of the other cancers attributable to the targeted HPVs. These other cancers may represent an additional 75,000 deaths per year worldwide that might be prevented by the current vaccine.

**Ovarian Cancer Prevention and Early Detection Study**

NCI, in collaboration with the Gynecologic Oncology Group and the Cancer Genetics Network, is funding a new, 5-year prospective study to identify ways to lower the risk of developing ovarian cancer as well as improve the ability to detect this cancer at an earlier, more readily curable, stage. This study targets women at elevated risk of developing ovarian cancer, either because they have a strong family history of breast and/or ovarian cancer or because they have tested positive for changes in genes that increase the risk of developing ovarian cancer. The study is evaluating two interventions: (1) risk-reducing removal of the ovaries and fallopian tubes; and (2) a novel ovarian cancer screening strategy. All study participants will complete the same set of questionnaires, receive an ultrasound examination of the ovaries, and provide blood samples. The latter will be used for biomarker development and validation. Women in each study group will be closely monitored for the occurrence of breast and ovarian cancer as well as other outcomes of interest. In addition, the protocol includes a detailed assessment of quality-of-life, medical decision-making, and the management of nononcologic complications of premature menopause. At present, this study is open at 90 sites across the United States, and it has accrued 460 (of a planned 1,800) subjects.

**Kidney Cancer Case-control Study**

In the years from 1973–1977 to 1988–1992, incidence rates of U.S. and international kidney cancer rose among men and women in all regions and ethnic groups, with a few exceptions, mostly in Scandinavian countries. The largest percentage increases were for men in Japan (171 percent) and for women in Italy (107 percent). In the years 1988–1992, kidney cancer incidence rates were highest in France and lowest in India. Rates for renal pelvis cancer were less than 1/100,000 person-years in almost all regions in both sexes, and the temporal trends were inconsistent.

In the United States, more rapid increases in the incidence and mortality rates for renal cell cancer have been noted among African Americans than Caucasians. However, the reasons behind the upward trend and the ethnic differences remain unclear. In April 2002, NCI scientists launched the "Case-control Study of Renal Cell Cancer Among Caucasians and African Americans in the U.S." This population-based study will strive to identify environmental and genetic determinants that underlie the demographic patterns. In particular, the investigators hope to clarify the role of smoking, obesity, hypertension, medications, susceptibility genes, and other factors in the etiology of renal cancer. The investigators plan to recruit 1,400 Caucasian and 700 African-American cases

and 2,800 controls from the Detroit and Chicago metropolitan regions over a 4-year period. Participants will complete a 90-minute interview and provide samples of saliva and blood.

**Bladder Cancer in the Northeastern United States**

Data from the new cancer atlas covering the period 1970–1994 indicate that bladder cancer mortality rates among white men and women are elevated in the northeastern United States, particularly in the northern parts of New England including Maine, New Hampshire, and Vermont. The reasons for these high mortality rates are unclear. The persistent elevations in mortality and incidence for bladder cancer among both men and women suggest the possible role of environmental factors. A leading suspect exposure is inorganic arsenic in drinking water, which is elevated in private wells in parts of New England. The purpose of this study is to determine the risk factors that explain the high mortality and incidence rates for bladder cancer in men and women in northern New England. The study will be a population-based case-control study of carcinoma of the urinary bladder in 3 states: New Hampshire, Vermont, and Maine. All histologically confirmed incident cases of carcinoma of the bladder occurring within a 3-year period among residents of the study areas between the ages of 30 and 79 years will be eligible for the study. Controls will be selected randomly from the general population of each study area, frequency matched to the age-, race-, and gender-specific distributions of incidence cases of bladder cancer in each state. We expect to interview 1,200 cases and 1,200 controls. Several data collection activities will be incorporated in this study: a self-administered residential/occupational history calendar; a self-administered diet questionnaire; in-person interviews with subjects using a computer-assisted personal interview (CAPI); collection of drinking water to determine levels of arsenic and other contaminants in drinking water; and collection of buccal cells, toenails, spot urine, 4-day urinary habits diary, overnight urine (from a sample of 240 cases and 240 controls), and blood (from a sample of 180 cases and 180 controls). Tumor tissue samples will be obtained for cases. We are examining associations between bladder cancer and environmental exposures in efforts to explain the elevated mortality and incidence in northern New England.

**Testicular Cancer among U.S. Servicemen**

The incidence of testicular germ cell tumors (TGCT) has increased during the better part of the twentieth century and is of particular concern as it primarily affects young men. Though the tumor is relatively infrequent in the population as a whole, TGCT is the most common cancer among U.S. males in the age group 25–34 years. Despite the increases in TGCT rates, the etiology is still poorly understood. The only well-described risk factors for TGCT are cryptorchism, family history of TGCT, and prior history of TGCT. Therefore, in order to understand better the environmental and genetic determinants of TGCT risk, NCI scientists are conducting a case-control study among members of the U.S. Armed Forces. The study includes men who have donated a blood sample to the Department of Defense Serum Repository (DoDSR) between the years 1989 and 2002. All DoDSR donors who have developed GCT are matched to DoDSR donors who have not developed TGCT. The DoDSR serum sample will be tested for levels of organochlorines, gonadotropin levels, and viral antibody titres. In addition, each participant is donating a current buccal cell specimen that will be used to examine genetic susceptibility. Physical activity, medical history, medication history, and other risk factors of the participants will also be analyzed. In addition, the mothers of all participants are being invited to complete a questionnaire concerning a variety of

possible risk factors such as physical activity, medical history, medication history, perinatal exposures, and other risk factors. The mothers are also being asked to donate a buccal cell specimen. As of November 2004, the study had enrolled 736 cases; 838 controls; and 1,048 mothers of the case and control men. The study is scheduled to cease field activities in February 2005.

**Breast/Ovarian Cancer Family Registries**

The Breast/Ovarian and Colon Cancer Family Registries (CFR) studies support research to identify genetic changes that predispose to breast, ovarian, and colon cancers, and to explore gene-gene and gene-environment interactions that may contribute to the development of cancer among families with these cancers. These registries provide the tools and resources needed to clarify gene-environment interactions in cancer risk. They have identified thousands of families at high risk for breast, ovarian, and colorectal cancers who have agreed to be part of this research. Of particular interest are potential collaborations aimed at identification and characterization of cancer susceptibility genes; definition of gene-gene and gene-environment interaction in cancer etiology; and cooperative research on the translational, preventive, and behavioral aspects of such findings. The outcome will be a clearer understanding of the genes that affect the development of cancer, and how environmental factors may modify these genes.

**Academic Public Private Partnership Program**

A new initiative, called the Academic Public Private Partnership Program (AP4), has been created to support the discovery of new cancer agents and their rapid translation to human clinical trials. With this program, the NCI will support collaborations between universities, pharmaceutical companies, biotech companies, and nonprofit organizations. A new funding mechanism that would accomplish this goal was called for by several Progress Review Groups. The effort began in July 2003 with the announcement of an RFA for a 1-year planning grant, which will be utilized by the proposed AP4 directors to bring together potential partners and to compose the center application. In July 2004, 14 planning grants were awarded, descriptions of which can be found at <http://dtp.nci.nih.gov/docs/ap4/ap4-index.jsp>. The AP4 initiative represents a new paradigm in drug discovery, development, and delivery for the NCI.

At the suggestion of the Leukemia, Lymphoma, and Myeloma Progress Review Group, NCI is supporting the formation of partnerships among academia, industry, nonprofit institutions, and government entities. These AP4s will research novel cancer therapeutic, prevention, diagnostic, and imaging interventions. The overall goal of the partnerships will be to speed the translation of newly discovered cancer interventions to clinical trials. The NCI is assisting the formation of these partnerships by funding 1-year planning grants.

The planning grants will be utilized by the principal investigators to study the feasibility of developing the pharmaceutical/nonprofit/academic interaction necessary to establish and support a partnership, to hold a meeting of potential partners, and to select research projects for the potential AP4 center. This effort is a two-step application process: only awardees of the planning grant are eligible to apply for the AP4 Center grant.

The principal investigators and institutions are recognized leaders in the field of anticancer intervention, discovery, and development and have assembled excellent teams to plan and organize the AP4 centers. The AP4 center grant applications, which will be the end product of these planning grants, will afford NCI an optimal opportunity to fund top-caliber, results-oriented research. Of the 14 potential partnerships currently being planned, two are focused on blood cancers.

Dr. Kit S. Lam, University of California, Davis Cancer Center, Davis, CA, is the codirector of a cancer therapeutics team consisting of 50 researchers who work collaboratively on three main themes: drug discovery, drug development, and clinical investigations. The potential AP4 center will concentrate on orphan cancers including acute myelocytic leukemia (AML), acute lymphocytic leukemia (ALL), and ovarian cancer. The team resides in an area abundant in institutes and corporations in fields relevant to drug discovery and development.

The AP4 center at Northwestern University proposed by Dr. Thomas O'Halloran focuses on the development of improved diagnostics and therapeutic for two rare and generally fatal malignancies, pancreatic cancer and multiple myeloma. Most pancreatic cancer patients have metastatic disease at the time of diagnosis and succumb to the disease within a year. While new therapies have extended survival times for multiple myeloma patients, the disease is still invariably fatal. Dr. O'Halloran plans to utilize the existing research strengths in the Northwestern University Chemistry of Life Processes Institute and in the Robert H. Lurie Comprehensive Cancer as the foundation for the new AP4 center. The research environment includes excellent chemistry, materials science, engineering, biology, and medicine disciplines. Investigators from clinical oncology, molecular biology, diagnostics, imaging, nanotechnology, and drug development will be brought together in this center. This potential AP4 center has an excellent opportunity to bring together institutional strengths, the expertise and resources of its pharmaceutical and biotechnology partners, and the support and input of patient advocacy organizations.

**Drug Development for Cancer, Including Rare Cancers**

NCI continues to screen new synthetic and natural compounds for antitumor activity using the automated cancer cell line screen. Over 87,000 defined chemical structures have been evaluated since the screen became operational in April 1990. More than 9,200 compounds have demonstrated *in vitro* antitumor activity, of which more than 5,200 agents have been selected for *in vivo* evaluation for assessment of therapeutic activity. Obviously, there are more compounds to test/develop than current resources would allow. The Drug Development Group (DDG) oversees the decision-making process regarding the development of new drugs and relies on extramural review of proposed activities. A complete description of this process is available on the Developmental Therapeutics Program Web site (dtp.nci.nih.gov). Including vaccines and other biologicals, as well as chemotherapeutic agents, 4 agents are in DDG level 1B (early preclinical testing), 9 are in DDG level 2A (GMP production and late preclinical testing), 6 agents are in DDG level 2B (IND-directed toxicology), and (need count from CTEP) are in DDG level 3 (ready for human testing subject to obtaining an IND). Table 1 lists the agents in the DDG process. As the agents move through the different levels of the decision process, the level of NCI’s financial commitment increases.

To expedite the movement of academic discoveries from the laboratory to proof-of-principle clinical trials, NCI initiated the program Rapid Access to Intervention Development (RAID) in 1998. RAID makes resources available, on a competitive basis, to the academic research community that are necessary to convert a new molecule into a drug candidate suitable for clinical testing and are generally not available to academic investigators who lack a corporate partner. These resources include: (1) GMP synthesis, formulation, range finding, and IND-directed toxicology and pharmacology; (2) clinical trial planning; and (3) regulatory assistance so that FDA requirements may be satisfied by any investigator who seeks to put a new molecule into the clinic.

As of December 2004, 288 applications have been received, 104 of which were approved for NCI support. A description of the successful applicants and the projects can be found at dtp.nci.nih.gov/docs/raid/raid_index.html. Table 4 lists current RAID projects pertaining to rare diseases.

**Table 1. Compounds That Passed Drug Development Group (as of December 2004)**

**Drug Development Group 1B**

| NSC Number | |
|---|---|
| 719239 | Discreet |
| 725088 | Discreet |
| 728930 | Discreet |
| 733269 | JMP |

**Drug Development Group 2A**

| NSC Number | |
|---|---|
| 680410 | Adaphostin |
| 656240 | Dithiophene and derivatives |
| 682994 | Dithiophene and derivatives |
| 703939 | RFB4-onconase |
| 711516 | Chimeric antiamyloidosis MAb |
| 722333 | Transferrin-doxorubicin conjugate |
| 724910 | Discreet |
| 721782 | 1-methyltryptophan |
| 722134 | Discreet |

**Drug Development Group 2B**

| NSC Number | |
|---|---|
| 281612 | Dimethane sulfonate |
| 309132 | Zebularine |
| 710464 | Aminoflavone |
| 711193 | CDDO |
| 713205 | Halofuginone |
| 729746 | HA22 |

**Drug Development Group 3**

| NSC Number | |
|---|---|
| 716976 | BNP7787 |
| 371331+112907 | Cytochlor + Tetrahydrouridine |
| 724770 | VEGF-Trap |
| 724636 | Biricodar (VX-710) |
| 724772 | BAY 43-9006 |
| 663249 | Triapine |
| 726292 | MLN 518 |
| 727990 | SB-715992 |
| 727989 | GW-572016 |
| 707545 | 17-DMAG |
| 701852 | SAHA |
| 728876 | 90Y-DOTA-Hpam4 |
| 729968 | Reolysin |

694501 Pyrrolobenzodiazepine (SJG136)
720735 Discreet (PPI-2458-[Fumagillin Analog]
703813 CC-5013
732208 AZD2171
733606 ABT472
732517 BMS 354825
710464 Aminoflavone
733504 RAD001
731636 SGN-30
711193 CDDO

**Table 2: Active Research and Development Agreements (as of December 21, 2004)**

The NCI cooperates on the development of novel anticancer therapies with commercial as well as institutional entities ranging from fresh startups to the multinational biopharmaceutical firms. NCI currently holds 36 Cooperative Research and Development Agreements (CRADAs), 52 Clinical Trials Agreements (CTAs), 12 Clinical Supply Agreements (CSAs), and 203 Material Transfer Agreements (MTAs) with its collaborators.

| Agent | Company | Type |
|---|---|---|
| 17-AAG | KOSAN BIOSCIENCES | CRADA |
| 17-DMAG | KOSAN BIOSCIENCES | CRADA |
| 280-446 | NOVARTIS | CTA |
| 2-METHOXYESTRADIOL (Panzem) | ENTREMED, INC. | CRADA |
| 506U78 (Nelarabine) | GLAXOSMITHKLINE | CTA |
| 5-AZACYTIDINE | PHARMION CORPORATION | CTA |
| ABT 472 | ABBOTT LABORATORIES | CTA |
| AE-941 | AETERNA | CTA |
| ALL-TRANS RETINOIC ACID | HOFFMANN-LAROCHE | CTA |
| ANTI-CTLA4 ANTIBODY | MEDAREX | CTA |
| Antigen genes formulated for delivery in a dermal powderject xr gene delivery device | POWDERJECT | CTA |
| ARSENIC TRIOXIDE (Trisenox) | CELL THERAPEUTICS, INC. | CRADA |
| ARTEMISININ | ELSOHLY LABORATORIES | M-CRADA |
| AZD2171 | ASTRAZENECA PHARMACEUTICALS LP | CRADA |
| BAY 43-9006 (Sorafenib) | BAYER CORPORATION | CTA |
| BENZOYLPHENYLUREA | ISHIHARA SANGYO KAISHA | CTA |
| BEVACIZUMAB (Avastin) | GENENTECH | CRADA |
| BMS 214662 | BRISTOL-MYERS SQUIBB | CTA |
| BMS 247550 (Ixabepilone) | BRISTOL-MYERS SQUIBB | CTA |
| BMS 275291 (MMPI) | BRISTOL-MYERS SQUIBB | CTA |
| BMS 354825 | BRISTOL-MYERS SQUIBB | CTA |
| BNP7787 | BIONUMERIK PHARMACEUTICALS | CTA |
| CAMPATH 1H (Alemtuzumab) | BERLEX LABORATORIES | CTA |
| CARBOXYPEPTIDASE G2 | PROTHERICS INC. | CTA |
| CCI-779 (Temsirolimus) | WYETH PHARMACEUTICALS, INC. | CRADA |
| CDDO | REATA DISCOVERY, INC. | CRADA-LOI |
| CILENGITIDE (EMD 121974) | MERCK KGAA | CRADA |
| CLODRONATE | SCHERING OY | M-CRADA |
| COL-3 (Medastat) | COLLAGENEX | CRADA |
| CYTOCHLOR, THU | HALOGENETICS, INC | CTA |
| DECITABINE | SUPERGEN, INC. | CRADA |
| E7389 | EISAI RESEARCH INSTITUTE | CRADA |
| EMD 273063 | EMD PHARMACEUTICALS | M-CRADA |
| EXEMESTANE | PFIZER | M-CRADA |

| | | |
|---|---|---|
| FK228 (Depsipeptide) | GLOUCESTER PHARMACEUTICALS | CRADA |
| FLAVOPIRIDOL (Alvocidib) | SANOFI-AVENTIS | CRADA |
| G3139 (Oblimersen sodium) | GENTA | CRADA |
| GADOLINIUM TEXAPHYRIN (Gd-Tex) | PHARMACYCLICS | CRADA |
| GM-CSF (Sargramostim) | BERLEX LABORATORIES | CTA |
| GTI-2040 | LORUS THERAPEUTICS | CTA |
| GW572016 (Lapatinib ditosylate) | GLAXOSMITHKLINE | CTA |
| HALOFUGINONE (Cebegine) | COLLGARD PHARMACEUTICALS | CRADA |
| HERCEPTIN (Trastuzumab) | GENENTECH | CRADA |
| HSP-E7 | STRESSGEN BIOTECHNOLOGIES | CTA |
| ID-KLH LYMPHOMA VACCINE | BIOVEST INTERNATIONAL | CRADA |
| IL-2 | CHIRON CORPORATION | CTA |
| ING201 | INTROGEN | CTA |
| IRESSA (ZD1839) | ASTRAZENECA PHARMACEUTICALS LP | CTA |
| IRINOTECAN (Camptosar) | PFIZER | CTA |
| KRN5500 | KIRIN | CTA |
| LUTETIUM TEXAPHYRIN (Lu-Tex) | PHARMACYCLICS | CRADA |
| MEDI-522 (Vitaxin) | MEDIMMUNE | CRADA |
| MGI 114 (Irofulven) | MGI PHARMA | CTA |
| MLN 518 | MILLENNIUM PHARMACEUTICALS | CRADA |
| MS-275 | SCHERING AG | CRADA |
| O6-BENZYLGUANINE | AOI PHARMACEUTICALS | CRADA |
| OSI-774 (Tarceva; erlotinib) | OSI PHARMACEUTICALS, INC. | CTA |
| OXALIPLATIN (Eloxatin) | SANOFI-SYNTHELABO | CRADA |
| PERIFOSINE | AOI PHARMACEUTICALS | CRADA |
| PPI 2458 (Fumagillin Analog) | PRAECIS PHARMACEUTICALS | CTA |
| PR-1 VACCINE | VACCINE COMPANY | CTA |
| PS-341 (Bortezomib; Velcade) | MILLENNIUM PHARMACEUTICALS | CRADA |
| PSC-833 (Cyclosporin) | NOVARTIS | CTA |
| PV701 | WELLSTAT | CRADA |
| PXD-101 | CURAGEN | CTA |
| PYRAZOLOACRIDINE | PARKE-DAVIS | CTA |
| R115777 (Tipifarnib, Zarnestra) | JOHNSON & JOHNSON R&D | CTA |
| RAD001 | NOVARTIS | CTA |
| REBECCAMYCIN ANALOG (Becatecarin) | EXELIXIS | CTA |
| REOLYSIN | ONCOLYTICS BIOTECH | CTA |
| REVLIMID (lenalidomide) | CELGENE CORPORATION | CTA |
| rF-TRICOM, rF-CEA-TRICOM | THERION | IntMurl-CRADA |
| RITUXIMAB | BIOGEN IDEC | CRADA |
| rV-B7.1 | THERION | IntMurl-CRADA |

| | | |
|---|---|---|
| SAHA | MERCK & CO. | CTA |
| SB-715992 | GLAXOSMITHKLINE | CTA |
| SC-55494 | SEARLE | CTA |
| SGN-30 | SEATTLE GENETICS | CTA |
| SJG-136 | IPSEN | CRADA |
| SMART 1D10 (HU1D10, Apolizumab) | PROTEIN DESIGN LABS, INC. | CTA |
| STI571 (Gleevec, Imatinib mesylate) | NOVARTIS | CRADA |
| THALIDOMIDE (Thalomid) | CELGENE CORPORATION | CTA |
| TIRAPAZAMINE (Tirazone) | SANOFI-SYNTHELABO | CTA |
| TOPOTECAN (Hycamptamine) | GLAXOSMITHKLINE | CTA |
| TRIAPINE | VION PHARMACEUTICALS | CTA |
| Tumor Necrosis Factor-alpha (Tasonermin) | BOEHRINGER INGELHEIM | CTA |
| UCN-01 | KYOWA HAKKO KOGYO | CTA |
| VEGF TRAP | SANOFI-AVENTIS | CTA |
| VX710 (Biricodar) | VERTEX PHARMACEUTICALS | CTA |
| XK469 | BRISTOL-MYERS SQUIBB | CTA |
| ZEVALIN (Ibritumomab tiuxetan) | BIOGEN IDEC | CTA |

**Table 3: Investigational New Anticancer Agents in Early Clinical Trials (as of January 2005)**

| Phase I | Phase II |
|---|---|
| **Biologic Agents** | |
| Advexin® (adenovirus p53) | Advexin® (adenovirus p53) |
| Anti-idiotype-KLH Myeloma Vaccine | Anti-idiotype-KLH Myeloma Vaccine |
| Antisense GTI-2040 | Antisense GTI-2040 |
| Apolizumab (MoAb: Hu1D10) | Apolizumab (MoAb: Hu1D10) |
| Apolizumab + Rituxan® | Avastin™ (bevacizumab, MoAb: anti-VEGF) |
| Autologous T cells + rF-gp100P209 | Avastin™ PAP-pulsed Dendritic Cells |
| Avastin™ (bevacizumab, MoAb: anti-VEGF) | BL22 Immunotoxin |
| BL22 Immunotoxin | Campath® [MoAb: CAMPATH-1H (Anti-CD52)] |
| Campath® [MoAb: CAMPATH-1H (Anti-CD52)] | CAP-1 peptide vaccine |
| gp 100 IVS Cells Vaccine | Expanded activated T cells + IL-2 |
| gp100TCR-transduced PBL + TIL | ESO-1 Peptides |
| gp100 Protein (184V) Vaccine | FGF-5:172-176/217-220 peptides |
| Genasense® (G3139 ASO) | Genasense® (G3139 ASO) |
| IL-2 transduced cells (retroviral vector) | gp100 IVS Cells Vaccine |
| IL-12 | gp100 Peptides A,C,F Vaccine |
| IL-12 + IL-2 | Herceptin® (trastuzumab; MoAb: humanized Her2) |
| LBM-2 Immunotoxin | IL-12 |
| MDX-010 (Human Anti-CTLA4 MoAb) | IL-12 + IL-2 |
| MoAb: 3A1, 95-5-49, 95-6-22 (Anti-T cell) | LMB-2 Immunotoxin |
| MoAb: HeFi-1 (Anti-CD30) | MDX-010 (Human Anti-CTLA4 mAb) |
| MoAb: I-131 HuCC49 Delta CH2 | MoAb: 3A1, 95-5-49, 95-6-22 (Anti-T cell) |
| MS275 | MSGV1AIB(anti-MART-1 TCR) Retroviral Vector-Transduced Autologous TIL/PBL |
| PANVAC™-V and PANVAC™-F | Mutated VHL Peptides Vaccine |
| PR-1 Peptide Vaccine | PAX3/FKHR/EWS/FLI1&2 Vaccine |
| Prosvac® (PSA-TRICOM vaccine) | PG13/LNc8 RetrovirusTransduced T cells |
| ras/p53 Vaccine | PR-1 Peptide Vaccine |
| Recombinant Fowlpox- CEA(6D)/TRICOM + Vaccinia- CEA(6D)/TRICOM Vaccine | Prosvac® (PSA-TRICOM vaccine) |
| Recombinant Fowlpox-TRICOM and Vaccinia-TRICOM Vaccine | ras/p53 Vaccine |
| Rituxan® (rituximab, MoAb: IDEC-C2B8)/Chemotherapy | Recombinant Fowlpox- CEA(6D)/TRICOM + Vaccinia- CEA(6D)/TRICOM Vaccine |
| SS1(dsFv)PE38 | Recombinant Fowlpox-PSA Vaccine |

| | |
|---|---|
| Sodium Phenylbutyrate (IV) | Recombinant Fowlpox-TRICOM and Vaccinia-TRICOM Vaccine |
| Vitaxin® (MEDI-522) | Rituxan® (rituximab, MoAb: IDEC-C2B8)/Chemotherapy |
| Zevalin® (MoAb: Y2B8) | SGN-00101 (HspE7) Vaccine |
| | Sodium Phenylbutyrate (IV) |
| | Thalidomid® (thalidomide) |
| | Zevalin® (MoAb: Y2B8) |

**Chemotherapeutic Agents**

| | |
|---|---|
| 17-AAG | 17-AAG |
| 17-DMAG | BAY 43-9006 |
| BAY 43-9006 | BMS 247550 (epothilone B analog) |
| BMS 214662 (FTI) | Bryostatin 1 |
| BMS 247550 (epothilone B analog) | CAI |
| BPU | CCI-779 (rapamycin analog) |
| Bryostatin 1 | COL-3 |
| CAI | Decitibine |
| Camptosar® (irinotecan, CPT-11) | Depsipeptide |
| CCI-779 (rapamycin analog) | EF5 |
| CC-5013 | Eloxatin® (oxaliplatin) |
| COL-3 | EMD 121974 |
| Cytochlor + Tetrahydrouridine | Fenretinide (4-HPR) |
| Decitibine | Flavopiridol |
| Depsipeptide | Gleevec® (imatinib mesylate, STI571) |
| E7389 (halichondrin B analog) | GW572016 |
| EF5 | Halofuginone (topical) |
| Eloxatin® (oxaliplatin) | Hycamtin® (topotecan) |
| EMD 121974 | Iressa® (ZD1839) |
| Fenretinide (4-HPR) | Irofulven (MGI-114) |
| Flavopiridol | MMPI (BMS 275291) |
| Gadolinium Texaphyrin | Nelarabine® (compound 506U78) |
| Gleevec® (imatinib mesylate, STI571) | $O^6$-Benzylguanine ($O^6$-BG) |
| Hycamtin® (topotecan) | Perifosine |
| Iressa® (ZD1839) | Pyrazoloacridine |
| Irofulven (MGI-114) | Rebeccamycin Analog |
| KRN5500 | SB-715992 |
| Lutetium Texaphyrin | Suramin |

| | |
|---|---|
| $O^6$-Benzylguanine ($O^6$-BG) | Tarceva® (OSI-774) |
| Panzem® [methoxyestradiol (2ME2)] | Taxotere® (docetaxel) |
| Pyrazoloacridine | Tirapazamine |
| Rebeccamycin Analog | Triapine® |
| SarCNU | Trisenox® (arsenic trioxide) |
| SB-715992 | UCN-01 |
| SJG136 | Velcade® (bortezomib, PS341) |
| Suramin | Zarnestra® (R115777) |
| Tarceva® (OSI-774) | |
| Taxol® (paclitaxel) | |
| Tirapazamine | |
| Triapine® | |
| Trisenox® (arsenic trioxide) | |
| UCN-01 | |
| Velcade® (Bortezomib, PS341) | |
| XK469 | |
| Zarnestra® (R115777) | |

**Table 4. Current RAID Projects for the Treatment of Rare Diseases (As of 12/04)**

| Compound NSC | Name | Disease | Investigator | Pediatric Use |
|---|---|---|---|---|
| 710296 | C-myb Antisense Oligodeoxynucleotide | Acute Myelocytic Leukemia | Dr. Alan Gewirtz; University of Pennsylvania, School of Medicine | Yes |
| 710292 | Lipopeptide | Cytomegaloviru s | Dr. Don Diamond; City of Hope Medical Center | Yes |
| 711518 | Allogenic Pancreatic Tumor Vaccine | Pancreas | Dr. Elizabeth M. Jaffee; Johns Hopkins University | |
| 711519 | IGF-1R Antisense Oligodeoxynucleotide | Glioma | Dr. Robert Aiken; Thomas Jefferson Medical College | Yes |
| 714597 | Imexon | Multiple Myeloma | Dr. Robert Dorr; University of Arizona, Arizona Cancer Center | |
| 113090 | Betulinic Acid | Multiple Myeloma | Dr. Tapas Das Gupta; University of Illinois at Chicago | |
| 734551 714503 | Fenretinide plus Safingnol | Neuroblastoma, Pancreas, Acute Leukemias | Dr. C. Patrick Reynolds; University of Southern California School of Medicine | Yes |
| 715815 | Chimeric Anti-CD54 monoclonal Antibody (UV3) | Multiple Myeloma | Dr. Ellen Vitetta; University of Texas, Southwestern Medical Center | |
| 715816 | Tropism-Modified Adenoviral Vector | Ovary | Dr. Glenn Peters; University of Alabama, Comprehensive Cancer Center | |
| 71887 | Psuedomonas Exotoxin Construct | Glioblastoma Multiforme, Neoplastic Meningitis | Dr. Darrell Bigner; Duke University Comprehensive Cancer Center | Yes |
| 719277 | Nonpathogenic Oncolytic Poliovirus Chimeras | Glioma | Dr. Matthias Gromeier; Duke University Medical Center | Yes |
| 720454 | vac-mTag Recombinant Vaccinia Construct | Mesothelioma | Dr. Harvey Pass; Barbara Ann Karmanos Cancer Institute | |
| 720836 | IL-6 plus Interferon | Multiple Myeloma | Dr. Richard Jones; Johns Hopkins University | |
| 722667 | Folate receptor-targeted liposomal daunorubicin (F-L-DAU) | Acute myelocytic leukemia | Dr. Robert Lee; Ohio State University | |

| | | | | |
|---|---|---|---|---|
| 723253 | Allogeneic multiple myeloma vaccine | Multiple myeloma | Ivan Borrello; Johns Hopkins University | |
| 723256 | d-24-RGD oncolytic virus | Chronic lymphocytic leukemia | Alfred Yung; University of Texas, M.D. Anderson Cancer Center | |
| 725178 | Targeted CRAd | Pancreatic adenocarcinoma | Selwyn Vickers; University of Alabama | |
| 726189 | SHetA2 | Ovary | Doris Benbrook; University of Oklahoma | |
| 326231 | BSO | Neuroblastoma | Patrick Reynolds, Children's Hospital LA | Yes |
| 374551 | Oral formulation of fenretinide | Neuroblastoma | Barry Maurer; Children's Hospital LA | Yes |
| 723254 | hsp70-targeted E7 recombinant DNA vaccine | Cervical | Connie Trimble; Johns Hopkins University | |
| 731413 | Ad5/3-delta24 | Ovary | Akseli Hemminki; Helsinki University Hospital | |
| 731414 | MV-NIS virus | Refractory multiple myeloma | Stephen Russell; Mayo Clinic | |
| 731442 | KSR antisense oligonucleotide | Pancreatic | Richard Kolesnick; MSK Cancer Center | |
| 733624 | Frondoside A | Pancreatic | Thomas Adrian, Northwestern School of Medicine | |

## Initiatives Planned for the Future

### Case-control Consortia

By 2008, consortial studies of the several major cancers will be completed or under way. The breast and prostate cohort consortia have already been created and funded, while consortial studies of brain cancers and lymphoma will be conducted and analyzed in the short term. Cancers of high lethality (e.g., pancreas, liver, and esophagus) will also be studied through consortia, and a proposed Request for Application for these ongoing consortia will be initiated in 2005. In the short term, all of these consortia will be developed and the studies initiated, and these studies will be conducted and analyzed by the end of the mid term. Consortia to study other cancers, including childhood cancers, will be initiated in 4–6 years and conducted and analyzed in 7–10 years. The order and priority will depend on emerging hypotheses from population and laboratory research. The established consortia will be utilized to initiate both etiologic studies and early detection biomarkers of cancer precursors to identify carcinogenic mechanisms and preventive interventions. These studies may be expanded based on outcomes from the integrated cancer biology initiative. Linking the consortia through standardized platforms developed by the bioinformatics strategic initiative will accelerate accurate data collection and analysis. Factors that significantly influence the risk of developing cancer will be essential elements for other strategic initiatives including clinical interventions; cancer health disparities; and prevention, early detection, and prediction.

### Trans-NCI R21 Pancreatic Cancer Program Announcement

Pancreatic cancer is a highly lethal disease marked by pain, anorexia, sleep problems, and weight loss. It has the worst prognosis among all of the major malignancies. Despite efforts over the past century, conventional treatment approaches such as chemotherapy, radiation, surgery, or combinations of these modalities have had little impact on the course of this disease. It is clear that a better understanding of the molecular biology and biochemistry of pancreatic cancer is urgently needed to effectively diagnose, prevent, and treat this malignancy. Estimates by the American Cancer Society (ACS) indicate 31,860 new cases and 31,270 deaths from pancreatic cancer for 2004. The Trans-NCI Pancreatic Cancer Program Announcement would support a variety of research areas across multiple disciplines. Brief descriptions of extramural research across the cancer continuum identified by NCI division are provided here.

Cancer Biology—NCI is interested in expanding research in the biology of pancreatic cancer. Examples of appropriate research areas include, but are not limited to, how variations in cells combine with the microenvironment in the development of pancreatic cancer, development of experimental models for human pancreatic cancer, and exploration of molecular pathways important in cancer biology, particularly those that could lead to novel targets for therapeutic development.

Clinical Chemoprevention Trials—NCI is interested in smaller phase II trials involving persons at risk for familial pancreatic cancer or patients with pancreatic intraepithelial neoplasia (PANIN) lesions. Such cohorts could be treated with a COXIB, FTI, statin, etc, and then be followed with endoscopic ultrasound. These smaller exploratory clinical trials could be complemented by

additional epidemiological or preclinical studies, designed to identify candidate surrogate biomarkers and/or candidate chemopreventive or dietary compounds.

Candidate Biomarkers—Biomarkers are needed to assess: (1) pancreatic cancer risk, i.e., factors modifying pancreatic etiologic events and/or exposures, (2) response modification, i.e., response to a putative chemopreventive drug or dietary factor, and (3) pancreatic cancer advancement or stage, i.e., ability to predict pancreatic cancer outcome.

Cancer Control and Population Sciences—Identify genetic combinations that lead to pancreatic cancer, identify 'new' environmental exposures that contribute to pancreatic cancer, determine the relationship between inflammation and pancreatic cancer, develop a biofluid-based test for pancreatic cancer, and determine what combination of 'two hits'—genetic and/ or environmental—are needed for pancreatic cancer to develop.

Cancer Treatment and Diagnosis—The Clinical Grants and Contracts Branch as well as the clinical trials sponsored by the Investigational Drug Branch and the Clinical Investigations Branch at CTEP would benefit greatly from grant support specifically directed toward: Development of early stage clinical trials in pancreatic cancer, translational research associated with early stage clinical trials in pancreatic cancer, imaging studies associated with clinical trials in pancreatic cancer, exploratory studies to identify and evaluate biomarkers (with associated assay development) to determine prognosis and predict response to therapy in pancreatic cancer, and correlative studies using specimens from multi-institutional prevention and treatment trials.

**Workshop to Explore Public-private Partnerships for Childhood Cancers**

To help assure that children do receive timely benefit from advances in cancer biology, NCI will sponsor a workshop in FY2005 to address opportunities for public-private partnerships to identify childhood cancer molecular targets and to exploit this new knowledge for therapeutic benefit. The recommendations from this workshop will guide NCI efforts in establishing appropriate partnerships and in defining the most efficient and effective ways to support research that will identify effective molecular therapeutics for children with cancer.

**Future Research in Blood Cancers**

NCI has reissued two Program Announcements, Quick Trials for Novel Cancer Therapies: Exploratory Grants (PAR-04-155) and Clinical Cancer Therapy and Prevention Research (PA-04-046). These initiatives currently have many projects related to different types of blood cancers and addressed the PRG’s recommendations to provide for molecular characterization of hematological malignancies and new and novel treatment and prevention strategies.

**New Myeloproliferative Disorders Initiative**

NCI cosponsored with the NHLBI the RFA (HL-04-034) titled “Cellular and Genetic Discovery toward Curative Therapy in Myeloproliferative Disorders (MPD).” This initiative will fund up to 12 new grants in FY2005.

## C. Rare Disease-specific Scientific Conferences, Symposia, or Workshops with Outcomes

## D. Activities Rare Diseases Patient Advocacy Groups to Stimulate Research

## E. Education Activities on Rare Diseases for the Researcher, Public, and the Health Care Provider Community

***(These three sections are combined since most meetings now include investigators, advocates, interested members of the public, and health care providers.)***

**Cancer Survivorship: Genetic Susceptibility and Second Primary Cancers: Dr. Lois Travis, held November 8–9, 2004**

Given the increasing survival of cancer survivors, it becomes imperative to characterize the late effects of successful cancer treatment. Second primary cancers are now the number one cause of death among long-term survivors of selected cancers. Although the research community has made great strides in describing the risk and temporal patterns of second primary cancers, the identification of susceptible patient subgroups has not been systematically addressed. If genetically susceptible subsets of populations can be identified, the opportunity to tailor cancer therapy in order to minimize serious late toxicity would result. Susceptibility can be conferred because of numerous inter-individual differences in carcinogen processing and elimination, including polymorphisms in DNA repair pathways, cell cycle regulation, and genetic variation in drug metabolism and response. The study of cancer survivors provides a special opportunity to investigate the mechanisms of carcinogenesis, since it is the only setting in which humans are deliberately exposed to carefully measured amounts of known carcinogens (chemotherapy and radiation).

The goal of the meeting was to comprehensively review the current state of knowledge in this area and to identify important areas for future research. When complete, the final workshop summary will be presented to the NCI Executive Committee, the Prevention Committee of ASCO, and the Outcomes Group of ASTRO.

**Global Increases in Esophageal Adenocarcinoma: Current Epidemiologic Research and Future Directions: Dr. Wong Ho Chow, to be held May 9–10, 2005, in Rockville, MD**

Esophageal cancer overall is a rare malignancy. However, incidence rates of esophageal adenocarcinoma have been increasing rapidly worldwide for the past three decades, while rates for esophageal squamous cell carcinoma have declined. In the United States, the increases in esophageal adenocarcinoma among white men have outpaced all other cancers, and the rates have surpassed those of squamous cell carcinoma of the esophagus since 1994. Substantial increases of esophageal adenocarcinoma also are observed among women and minorities.

In the 1990s, several epidemiologic studies were launched in the United States and other countries to examine reasons for the upward trends of esophageal adenocarcinoma. These studies have provided convincing evidence linking risk of this cancer to smoking, obesity, and gastroesophageal reflux disease. However, the role of other potential environmental and genetic risk factors is still unclear. Major limitations were the relatively small number of cases that each study was able to recruit and the even fewer numbers of patients who provided biological samples for molecular

studies. The opportunities for more in-depth analyses of potential risk factors, particularly for exposures of relatively low prevalence and analyses of genetic and tumor markers, will be enhanced by pooling data from these investigations. NCI can play a valuable role by facilitating scientific dialogue, promoting the sharing of resources, and mapping future directions of interdisciplinary research in the etiology of this emerging cancer.

Proposed content/scientific issues: The proposed workshop will review current research, including data and type of biological samples collected in each study and findings to date, and discuss the feasibility and logistics for pooling the questionnaire data and biological samples. Participants will also discuss the future directions and type of epidemiologic studies needed to further elucidate the etiology and biological mechanisms leading to esophageal adenocarcinoma. To this end, the workshop will include presentations on state of the science laboratory technology that can be applied in epidemiologic investigations, including high-throughput genotyping, microarray, and proteomic analyses.

Anticipated goals: It is anticipated that the workshop will result in a preliminary plan for sharing data and resources among the existing studies and initiation of one to two proof-of principle analytical projects using the shared data. It is also anticipated that the workshop will stimulate ideas for future research.

**CLL Research Consortium Annual Meeting:**

The CLL Research Consortium had its annual meeting in Bethesda, MD, in April 2004. Members of the public, professional societies, and advocacy groups as well as staff of NCI and NHLBI were invited to attend.

**Natural History and Treatment of Peritoneal Mesothelioma:**

This meeting held September 13–14, 2004, in Bethesda included almost 200 participants including representatives from the Office of Rare Diseases and NCI faculty. Extramural faculty included U.S. and international investigators. Meeting sections included laboratory investigation, epidemiology, treatment, and outcomes.

**Other Meetings Include the Following:**

- 19th International Pigment Cell Conference "A Focus on Human Pigmentary Disorders"
- Linking Haplotypes and Genetic Variation with Cancer Risk Assessment, Prevention, Detection, and Treatment Workshop
- Sarcoma and Mesenchymal Stem Cell Biology Workshop
- Collaborative Approaches to Discovering Genes in African Americans and Hispanics
- Utilizing Mapping by Admixture Linkage Disequilibrium
- Lymphangiogenesis and Cancer: Research Directions and Therapeutic Opportunities
- Childhood Cancer Survivorship: Improving Care After Treatment

- RNA Interference-Target Validation and Potential Therapeutic Applications for Childhood Cancers
- Bloom Syndrome: Molecular Basis of Genomic Instability
- Genetic Diseases Caused by ABC Transporters
- Workshop on Chronic Graft-Versus-Host Disease
- FASEB Conference on Modulatory Adhesion Molecules in Tissue Organization

**Rare Diseases Research Activities and Advances**

*Center for Cancer Research, 2004*

**Molecular Signature Identified for Liver Cancer**

- A molecular signature has been identified that can be used to predict metastatic hepatocellular cancer (HCC) and patient prognosis. This signature can separate HCC patients into metastatic and nonmetastatic groups and may be useful for diagnosing HCC patients with metastatic potential. Currently, we are conducting a cross-validation study with a larger number of independent HCC patients to refine this signature. A serum profile has been established by determining the serum concentration of osteopontin and matrix metalloproteinase 9 to predict HCC patient survival and metastasis. Both microarray and serum profiling may provide a cross-validation of the metastasis prediction model in preparation for whether it should be recommended for aiding future clinical diagnosis.

**Clinical Trials in Pediatric Sarcoma and Leukemia Patients**

- The Pediatric Oncology Branch (POB) emphasizes clinical trials in high-risk sarcoma and leukemia patients and new drug development studies for pediatric cancer patients. Current efforts in high-risk sarcoma patients include development of effective immunotherapeutic strategies and utilization of allogeneic bone marrow transplant to generate graft-versus-tumor effects. Similar strategies are being used for recurrent leukemia patients. In addition, we are currently evaluating specific immunotoxin therapy for recurrent leukemias.
- The value of dynamic MRI imaging in predicting response to neoadjuvant chemotherapy is being studied in newly diagnosed osteosarcoma. The predictive value appears to be promising. Preliminary analysis of gene expression profiling on these tumors suggests that profiles may cluster patients into “good” and “poor” prognosis prospectively.
- IRB approval was recently received for a new salvage therapy for recurrent osteosarcoma and Ewing’s sarcoma using a combination of sequential gemcitabine/docetaxel based on preliminary *in vitro* synergy and previous activity of the single agents. This study will be performed through a newly formed sarcoma consortium and is supported by Aventis and Lilly.
- Current research efforts in the POB include clinical trials of novel, nonmyeloablative allogeneic stem cell transplant regimens for childhood hematopoietic malignancies. Biologic correlative studies conducted as part of these trials include valuation of chemotherapy-induced immunosuppression, immune recovery after allogeneic transplantation, and the pathophysiology of graft-versus-host disease. Additional clinical trial activities include phase I development of immunotoxins for refractory leukemias and lymphomas. In an effort to improve understanding of the molecular mechanisms of leukemogenesis and identify potential

new therapeutic targets, collaborative investigations of gene expression profiles of pediatric leukemias are also being conducted**.**

**Molecular Markers of Ewing's Sarcoma and Rhabdomyosarcoma Aid Diagnosis**

- Many pediatric solid tumors exhibit fundamental cytogenetic abnormalities that have implications in their pathogenesis. The Ewing's sarcoma family of tumors (ESFT) and alveolar rhabdomyosarcoma (RMS) are characterized by consistent chromosomal translocations that result in the fusion of genes and subsequent formation of novel chimeric genes. These molecular markers can be detected by RT-PCR or fluorescence in situ hybridization (FISH) and can be used not only to establish the diagnosis in difficult cases but also to understand the pathogenesis of these tumors. Recently, the products of these fusion genes have become the target of vaccine therapies in newly established protocols in the POB at the NCI.

**Novel Therapies Devised for Brain and Spinal Cord Tumors**

- Novel experimental therapeutics are being developed for children and adults with tumors of the brain and spinal cord. Toward this end, the translational laboratory efforts of the Neuro-Oncology Branch are focusing on new strategies for selective tumor targeting through gene transfer using neural and endothelial stem cells and novel genetic vectors, through the identification of tumor-selective processes such as angiogenesis, and through the identification of tumor-specific markers.
- Angiogenesis inhibition is being explored as a novel therapeutic strategy for the treatment of malignant gliomas. Selective delivery of genes of choice to tumor-associated angiogenesis can result in subsequent destruction of the tumor vascular bed. This novel strategy will be brought to the clinic soon.
- We are also in the process of identifying unique proteins expressed on infiltrating glioma cells and on endothelium, associated with the angiogenic response of tumors, for the purpose of identifying novel peptides and single chain antibody molecules. These molecules will be tagged with radioisotopes for diagnostic purposes and conjugated with immunotoxins.

**Gene Expression Profiling Predicts Outcome in Rhabdomyosarcoma and Neuroblastoma**

- The POB has developed substantial efforts in both proteomic and genomic characterization of pediatric tumors. The POB has carried out reverse-phase protein arrays on a set of 34 rhabdomyosarcomas. Expression of several proteins was highly associated with a high risk for relapse. Gene expression profiling is being performed on a series of neuroblastomas of different stages and prognosis to identify tumor-specific expression patterns or "fingerprints." The analysis has identified patterns of gene expression that accurately predicted outcome. These studies are also continuing in an attempt to identify new potential targets for therapy in poor-outcome patients.

**Microarray for Neurofibromatosis Developed to Identify Molecular Targets**

- The NCI POB is developing a tissue microarray for pediatric solid tumors and neurofibromatosis type 1 (NF1)-associated tumors with the goal of analyzing these tumors for the presence of targets of new molecularly targeted agents. The microarray will be used to make rational decisions in the drug development for pediatric cancers and NF1.

**Molecular Signature of Malignant Gliomas Sought**

- We are in the process of constructing a glioma-specific cDNA microarray chip to develop a molecular classification scheme of malignant gliomas. Through the identification of signature gene expression profiles, we hope to be able to group pediatric and adult gliomas into tumors that are biologically more related. This will allow us to offer more accurate prognoses and begin to address the issue of individualized therapies.

**Metastasis and Therapy Studied in New Mouse Models of Metastatic Osteosarcoma**

- The POB has a major focus on osteosarcoma. We have recently developed mouse models of metastatic osteosarcoma and identified novel proteins that appear to play a major role in metastatic behavior in this model and, hopefully, in human osteosarcoma as well. Work is ongoing to determine what signaling pathways mediate these effects on metastatic behavior with the hope that novel therapeutic interventions can be identified and tested for patients with metastatic osteosarcoma. We are also testing novel anti-metastatic agents using this mouse model.

**Artificial Neural Networks Predict Clinical Outcomes in Neuroblastoma**

- Researchers at the National Cancer Institute have used artificial neural networks (ANNs) and DNA microarrays to successfully predict the clinical outcome of patients diagnosed with neuroblastoma (NB). The ANNs also identified a minimal set of 19 genes whose expression levels were closely associated with this clinical outcome.
- Using the 19 predictor genes, the ANNs were also able to partition the subset of patients classified as high risk into good and poor outcome groups as well as predict which of the high-risk patients would fail conventional therapy. This has major clinical implications since we are now able to distinguish a group of ultra-high-risk patients who will not respond to conventional therapy and therefore require alternative treatment strategies. We may also be able to reduce the intensity and thereby reduce the toxicity of treatment regime to those predicted to survive based on their gene expression profile.
- We can translate our findings to the clinic because simple prognostic assays can be developed based on this small number of genes. In fact, three of the genes found to be overexpressed in poor-outcome tumors encode proteins secreted into the blood, meaning they could be used as serum prognosis markers in a simple blood test. In collaboration with industry, we are now developing clinical assays based on these 19 genes and planning to test for the presence of these serum markers in other patients with NB for prognostic prediction.

## Targeted Treatment Agents for Neurofibromatosis Tested in Clinical Trials

- The ras family of G-proteins plays an important role in the transduction of signals that trigger cell proliferation, and mutations in ras genes are found in 30 percent of all human cancers. Ras proteins undergo post-translational farnesylation, which is required for activity of wild-type and mutant ras proteins, and this step can be inhibited by farnesyltransferase inhibitors such as R115777. The evaluation of R115777 in children with refractory solid tumors and neurofibromatosis type I (NF1) is therefore a rational choice. A phase I trial of R115777 for children with these tumors was recently completed, and based on the results of this phase I trial, a multi-institutional, randomized, double-blinded, placebo-controlled, cross-over phase II trial of R115777 for patients with NF1 and progressive plexiform neurofibromas was developed and is open for patient accrual.
- A phase I trial of the antifibrotic agent pirfenidone is coordinated by the NCI, POB in collaboration with Children's National Medical Center, Washington, DC, and the Mayo Clinic, Rochester, MN, and just completed accrual. Subsequently a phase II trial of pirfenidone for children and young adults with NF1 and progressive plexiform neurofibromas was developed at the NCI, POB, and this trial just opened for accrual.

## Gene Expression Profiling Diagnoses Benign versus Malignant Thyroid Tumors

- Classification of human cancers into distinct groups based on their molecular profile rather than their histological appearance may prove to be more relevant to specific cancer diagnoses and cancer treatment regimes. Therefore, in this study, we developed a gene expression approach to diagnose benign versus malignant thyroid lesions in 73 patients with thyroid tumors. We successfully built a 10- and 6-gene model able to differentiate benign versus malignant thyroid tumors. Our results support the premise that a molecular classification system for thyroid tumors is possible, and this in turn may provide a more accurate diagnostic tool for the clinician managing patients with suspicious thyroid lesions.

## A Functional Polymorphism in *RGS6* Modulates Bladder Cancer Risk

- RGS proteins negatively regulate G protein signaling. Recent reports have shown that RGS proteins modulate neuronal, cardiovascular, and lymphocytic activity, yet their role in carcinogenesis has not been explored. In an epidemiologic study of 477 bladder cancer patients and 446 matched controls, three noncoding single-nucleotide polymorphisms (SNPs) in *RGS2* and *RGS6* were each associated with a statistically significant reduction in bladder cancer risk. The risk of bladder cancer was reduced by 74 percent in those individuals with the variant genotype at all three SNPs. In addition, we demonstrated that RGS2 transcripts and several splice variants of RGS6 are expressed in bladder cancer cells. These data provide the first evidence that RGS proteins may be important modulators of cancer risk and validate RGS6 as a target for further study.
- In an ongoing hospital-based case-control study, we explored the association between 12 noncoding SNPs in five *RGS* genes identified in the National Center for Biotechnology Information (NCBI) database (dbSNP) and the risk of bladder carcinoma, a cause of over 12,000 deaths *per annum* in the United States. Our results suggest that selected RGS variant SNPs may be important modifiers of cancer risk. To validate the biological significance of

these SNPs, we also sought to identify functional changes in transcript levels, alternative splicing events, and protein translation efficiency that may result from the presence of the variant alleles.

**Novel RNA-based Antiviral Therapies Devised for Cervical Cancer**

- The papillomaviruses are epitheliotropic small DNA tumor viruses that cause both benign and malignant lesions in humans and animals. An oncogenic subset of the human papillomaviruses (including HPV-16, -18, -31, and -33) is associated with the vast majority of cervical cancers as well as squamous cell carcinomas in other locations. Expression of papillomavirus genes is regulated at both transcriptional and post-transcriptional levels. We are currently exploiting HPV alternative splicing to develop novel RNA-based antiviral and anticancer therapies. In addition, we have been involved in several collaborations on HPV transcriptional and post-transcriptional regulation. Some of the techniques we have developed, such as isoform-specific real-time quantitative RT-PCR (QRT-PCR), are also being used in other collaborations both within and outside the papillomavirus field.
- Targeting HPV-infected cells using specific trans-splicing: The ideal cancer therapy would kill cancer cells and have no effect on normal cells. The expression of spliced human papillomavirus E6/E7 pre-mRNAs by the vast majority of cervical cancers provides an absolute difference between cancer cells and normal cells that can be exploited for the specific targeting of these cells. Through a CRADA between NCI and Intronn, Inc., we are investigating the use of Intronn's Spliceosome Mediated RNA Trans-splicing (SMaRT) Technology to develop a novel RNA-based suicide gene therapy for cervical cancer.

**Vaccines Developed Against HPV Virion Proteins**

- There is a strong association between malignant progression of human genital lesions and certain human papillomavirus (HPV) types, most frequently HPV-16. Our research is concerned with development of vaccines against HPV and other targets and elucidation of the PV life cycle. We have generated virus-like particles (VLPs) for HPV-16 and other PVs that consist of the L1 major capsid protein or L1 plus L2, the minor capsid protein. Parenteral injection of purified VLPs induced high titers of neutralizing antibodies and protection from experimental challenge in animal models. Based upon these results, we have validated GMP grade VLPs and have completed phase I and phase II clinical trials of an HPV-16 L1 VLP vaccine. Vaccines, even those vaccinated in the absence of adjuvant, consistently produced high titers of HPV-16 psuedovirion-neutralizing antibodies and reported only minor side effects. We are also developing alternative vaccine candidates, some for use in disease induced by HPV and others for use in diseases unlinked to HPV infection.

**Molecular Genetics of Gynecologic Cancers Characterized**

- Gynecologic cancer remains a major health problem for women in this country with approximately 25,000 deaths annually attributed to this problem. The purpose of this project is to characterize the molecular genetics of this group of tumors and ultimately use that information for clinical application in rationally designing therapeutic and prevention trials. We have characterized endometrial, cervical, and ovarian specimens that span the histiologic

spectrum from benign to malignant for mutations in the ras, p53, cyclin dependent kinase inhibitors, FHIT and Rb genes, and microsatellite instability. These studies should help to identify the molecular genetic events that are important in the genesis of endometrial and cervical cancers and their use for their early detection.

- Evaluation of ovarian tumors revealed that activated ras genes are found in benign (10 percent) and low malignant potential (LMP) (30 percent) tumors but not ovarian carcinomas (5–10 percent). In addition, mutations in the tumor suppressor genes p53 and Rb occur in ovarian carcinomas (48 and 14 percent respectively) but are not present in LMP tumors. This suggests that ovarian carcinoma and LMP tumors are discrete biologic entities.
- To identify potential new markers of ovarian cancer and genes important in its pathogenesis, malignant ovarian epithelium is being compared to its benign counterpart using differential display technology, representational display, and microarrays. As part of the Director's Challenge collaborative grant between this laboratory and MSK, 400 ovarian cancer specimens will be profiled utilizing cDNA microarrays and patterns will be correlated with clinical characteristics such as survival, histology, and stage. Genes that are differentially expressed will be isolated, cloned, and characterized for their roles in the development of ovarian cancer.

**Genome Expression Profiling of Ovarian Cancer Reveals Activated Pathways**

- Ovarian cancer is the most lethal type of gynecologic cancer in the Western world. The high case fatality rate is due in part because most ovarian cancer patients present with advanced stage disease that is essentially incurable. In order to obtain a whole genome assessment of aberrant gene expression in advanced ovarian cancer, we used oligonucleotide microarrays comprising over 40,000 features to profile 37 advanced stage papillary serous primary carcinomas. We identified 1,191 genes that were significantly ($P < 0.001$) differentially regulated between the ovarian cancer specimens and normal ovarian surface epithelium. The list of differentially expressed genes includes ones that are involved in cell growth, differentiation, adhesion, apoptosis, and migration. Based on our expression results, a signaling pathway associated with tumor cell migration, spread, and invasion was identified as being activated in advanced ovarian cancer.

**Recombinant Immunotoxins Developed for Ovarian and Pancreatic Cancer Therapy**

- Mesothelin is a differentiation antigen present on normal mesothelial cells and overexpressed in several human tumors, including mesothelioma and ovarian and pancreatic adenocarcinoma. Mesothelin is a promising candidate for tumor-specific therapy, given its limited expression in normal tissues and high expression in several cancers. We have developed a recombinant anti-mesothelin immunotoxin (SS1P) that targets ovarian cancers, mesotheliomas, and pancreatic cancers and is currently in clinical trials. Recombinant immunotoxin SS1P shows significant activity in preclinical models including complete regression of mesothelin-expressing tumor xenografts in mice as well as cytotoxic activity *in vitro* against tumor cells obtained directly from patients with ovarian cancer and peritoneal mesothelioma. We are using animal models to evaluate therapies that could enhance antibody delivery, with the goal of translating these findings to the clinic. Two phase I trials with immunotoxin SS1P are now open.

**Targeted Systemic Radiotherapeutics Evaluated for Intraperitoneal Cancers**

- Evaluations are being performed of the therapeutic efficacy of targeted systemic radiotherapeutics for treating metastatic intraperitoneal cancer, such as pancreatic cancer, while simultaneously optimizing their value in conjunction with chemotherapeutics. The majority of targeted ratation therapies have employed a single administration of a single isotope, while in fact, dose fractionation and combination therapies with other modalities clearly provide superior results. Studies combining the use of targeted α-emitters with chemotherapeutics are novel and unique to the NCI.

**Cytogenetic Signature of Vaginal Squamous Cell Carcinoma Identified**

- We used comparative genomic hybridization to establish a pattern of genomic imbalances in vaginal squamous cell carcinomas. Analysis of 16 tumors revealed that 70 percent of vaginal carcinomas carry relative copy number increases that map to chromosome arm 3q. Other recurring gains were observed on chromosome arms 5p and 19p. Chromosomal losses were infrequent. The cytogenetic data were related to the presence of human papillomavirus genomes, expression of laminin-5 as a marker for invasiveness, and expression levels of markers for proliferative activity and mutated *TP53*. The results suggest that vaginal carcinomas are defined by a specific distribution of chromosomal aneuploidies and that the pattern of genomic imbalances is strikingly similar to that observed in squamous cell carcinomas of the uterine cervix. Age at diagnosis, tumor size, and increased laminin-5 expression have a significant influence on the survival time. As in other malignant diseases, early detection greatly affects survival rates. It is therefore a perceived goal to analyze and understand the genetic mechanisms leading to vaginal tumorigenesis.

**Techniques Evaluated for Early Detection of Esophageal Cancer**

- Esophageal cancer has a very poor prognosis, primarily because most tumors produce symptoms only after they have spread beyond the esophageal wall and are unresectable. Significant improvement in survival will require successful strategies to diagnose and treat more cases at earlier, more curable stages of the disease. A project is being carried out in Linxian, China, a county with very high rates of esophageal cancer, to evaluate techniques that may be useful in a practical early detection and treatment program.
- The project includes five studies: the Cytology Sampling Study, the Mucosal Staining Study, the Endoscopic Staging Study, the Endoscopic Therapy Pilot Study, and the Chemoregression Study.

**Molecular Genetics of Kidney Cancer Studied**

- In order to identify the genes that cause kidney cancer and develop molecular therapeutics for this disease, we have studied the inherited forms of cancer of the kidney. Individuals with the inherited form of kidney cancer associated with von Hippel Lindau (VHL) are predisposed to develop tumors in the brain, spine, eyes, pancreas, adrenal gland, and inner ear. By studying VHL families we were able to perform genetic linkage analysis to localize and subsequently

identify the VHL gene on chromosome 3. We have identified the VHL gene mutation in the germline of 246/246 VHL kindreds and are currently studying genotype/phenotype relationships as well as evaluating minimally invasive forms of therapy for kidney and adrenal tumors in VHL. The VHL gene has been shown to be the gene for the hereditary form of renal carcinoma associated with VHL as well as the common form of sporadic (nonhereditary) kidney cancer (clear cell renal carcinoma).

**Angiogenesis in Renal Cell Cancer Targeted in Laboratory and Clinical Studies**

- Renal cell cancer (RCC) is a highly vascular tumor that contains a mutation in the VHL tumor suppressor gene in over 80 percent of clear cell carcinomas. This mutation is linked to deregulation of vascular endothelial growth factor (VEGF), a potent angiogenic agent. We are currently beginning a new initiative directed at identifying the mediators of angiogenesis and growth of RCC and neutralizing these biological activities in preclinical tumor models and in patients with RCC. In preclinical models of RCC xenografts in nude mice, we are evaluating neutralizing antibodies to FGF-5. RCC was found to express FGF-5 when expression screening of a cDNA library from an RCC line revealed FGF-5 as the targeted RCC-associated tumor antigen. This molecule, originally identified by its ability to transform 3T3, is expressed in approximately 60 percent of RCC lines and some prostate and breast cancer lines. We have not found it expressed by normal adult tissues by RT-PCR, and it therefore represents an excellent therapeutic target for multiple adenocarcinomas including RCC.
- Parallel to these laboratory studies, we are conducting clinical protocols of anti-angiogenic agents. In a new, accruing clinical protocol, we are evaluating the efficacy of a humanized neutralizing antibody to hVEGF (Genentech, Inc.) in a randomized, double-blind, placebo-controlled format in patients with progressive, metastatic RCC. In this 150-patient trial, we are also evaluating the use of novel noninvasive imaging techniques to evaluate anti-angiogenic agents in clinical trials. The next generation of clinical studies will be combinations of agents if the anti-VEGF monoclonal antibody demonstrates any significant clinical activity.

**Gene Expression Profiles Provide Diagnosis and Prognosis of Lymphoid Malignancies**

- To provide a molecular basis for the diagnosis of human lymphoid malignancies, we are exploiting DNA microarray technology to profile gene expression in these cancers on a genomic scale. The laboratory created a novel DNA microarray, the "Lymphochip," which is enriched in genes that are expressed in and/or function in lymphocytes.
- We have used Lymphochip and Affymetrix microarrays to profile gene expression in diffuse large B-cell lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma, multiple myeloma, and in a wide variety of normal lymphoid subsets.
- One central goal of these studies is to relate gene expression to clinical outcome, thereby establishing a quantitative, reproducible, and informative molecular diagnosis of the lymphoid malignancies. Our studies have revealed previously unknown types of diffuse large B-cell lymphoma that are indistinguishable by current diagnostic methods but have strikingly distinct gene expression profiles, originate from different stages of B-cell differentiation, utilize distinct oncogenic mechanisms, and differ in their ability to be cured by current chemotherapy. For several lymphoid malignancies, we have identified molecular profiles that predict the length of survival or the ability to be cured by chemotherapy, thereby providing clinically

useful prognostic indicators. Our laboratory has mounted a major effort to create a diagnostic microarray that could provide these molecular diagnoses and prognoses to patients with lymphoid malignancies.

- Importantly, the genes that are associated with clinical prognosis have provided new targets for therapy of the lymphoid malignancies. Our laboratory uses functional genomics, chemical genetics, and molecular biological techniques to validate these and other molecular targets toward the ultimate goal of targeted therapies for patients aimed directly at the disordered regulatory biology of their individual tumors.

**IGF-I Induces Migration and Invasion of Multiple Myeloma Cells**

- Multiple myeloma (MM) is an incurable form of cancer characterized by accumulation of malignant plasma cells in the bone marrow. During the course of this disease, tumor cells cross endothelial barriers and home to the bone marrow. In later stages, myeloma cells extravasate through blood vessels and may seed a variety of organs. Insulin-like growth factor I (IGF-I) is one of several growth factors shown to promote the growth of MM cells. In the current study, we have assessed the ability of IGF-I to serve additionally as a chemotactic factor affecting the mobility and invasive properties of these cells. Results indicate that IGF-I promotes transmigration through vascular endothelial cells and bone marrow stromal cell lines. The identification of IGF-I as both a proliferative and migratory factor provides a rational basis for the development of targeted therapeutic strategies directed at IGF-I in the treatment of MM.

**Novel Therapeutic Strategies for Lymphoma Assessed in Clinical Trials**

- *In vitro*, overexpression of the mdr-1 gene product, P-glycoprotein (Pgp), in tumor cells can confer high-level resistance to natural product-derived cytotoxics. It has been reported that Pgp was detectable by immunohistochemistry in 1/49 (2 percent) of untreated but was detectable in 6/8 (75 percent) treated lymphomas, suggesting that Pgp conferred drug resistance. To test this hypothesis, we developed and tested a mdr-1 reversal strategy in relapsed lymphomas using EPOCH (doxorubicin/vincristine/etoposide, prednisone, cyclophosphamide) and dexverapamil. Based on our results showing EPOCH to be effective and well tolerated, we began a phase II study of EPOCH in previously untreated patients with aggressive lymphomas. In this study, EPOCH doses are escalated within patients to the maximum tolerated dose (MTD). End points are dose intensity, efficacy, toxicity, and molecular markers of drug resistance. Early results show a high complete response rate of 89 percent with an EFS of 77 percent at 2 years median follow-up. Accrual continues to this trial.
- We have recently developed and tested a “second generation” EPOCH regimen (EPOCH II) to replace stem cell transplant for lymphomas requiring high-dose intensity including poor prognosis untreated aggressive lymphomas, potentially curable relapsed lymphomas, and low-grade lymphomas. This regimen is based on experimental/clinical observations that suggest infusion schedules may improve the therapeutic index of natural product-derived cytotoxics and that high-dose alkylator therapy can overcome drug resistance in lymphoma. Preliminary results show EPOCH II to have a response rate of 90 percent with 66 percent complete.
- Examination of tumor tissues to assess potential mechanisms of drug resistance is an ongoing effort in our group. We have demonstrated the association of clinical drug resistance with p53 mutation and low proliferation rate in relapsed lymphomas. Currently, we are studying other

novel cell-cycle proteins such as p27 in lymphomas. Recently, we have been interested in testing novel protein kinase inhibitors in lymphomas. One such inhibitor, UCN-01, has shown marked synergy with fludarabine in a variety of human cell lines. To further assess this drug, we will soon begin a phase I study of UCN-01 and fludarabine in patients with relapsed and refractory indolent lymphomas.

## NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT (NICHD)

### Overview of Rare Diseases Research Activities

The National Institute of Child Health and Human Development (NICHD) mission is to ensure that babies are born wanted, timely, and healthy and that they develop to their full physical, emotional, and cognitive potential. The Institute achieves its mission in part by conducting and supporting a broad range of innovative research activities, a portion of which specifically addresses rare diseases and conditions. These activities not only yield significant scientific advances but also provide a means to fill research gaps and better understand the origins of rare diseases and conditions. Below are some of the NICHD's activities related to possible ways to prevent or treat rare diseases and conditions as they affect children, women, and families.

### Recent Scientific Advances and Related Activities

*Study identifies novel puberty gene.* The mechanisms that control sexual maturation heralding the onset of adult reproductive function are one of the great mysteries of human biology. Puberty begins when a brain structure known as the hypothalamus begins secreting gonadotropin-releasing hormone, the "master" reproductive hormone that regulates fertility. However, NICHD-supported researchers came a step closer to understanding the maturation process when they examined several members of a family who did not experience normal sexual maturation. The researchers found that all of these individuals, along with another, unrelated person, had abnormalities in a gene known as GPR54. The researchers then developed an experimental mouse that did not have the gene in any form.[2] Like their human counterparts with mutations in GPR54, the mice also did not undergo puberty. The researchers found that while GnRH was lacking in its other tissues, the mouse hypothalamus did contain normal amounts of unreleased GnRH. These findings open the door to new ways of treating the ovaries and testes that fail to mature and other conditions of human infertility associated with inadequate release of GnRH.

*Rett syndrome protein involved in early development.* Rett syndrome (RTT) is a genetic disorder that gradually halts the healthy development of infant and toddler girls. Among other problems, girls with RTT lose their ability to talk, to interact with other people, and to move independently. Currently, no treatment exists to halt its progression. Researchers have determined that the disorder results from a defect in a particular gene, known as MeCP2, but were unsure of the gene's function. Recently, scientists gained an understanding of the gene's function by studying the underwater frog, *Xenopus*. The researchers determined that a mutant form of the gene affects early embryonic development, resulting in an excess number of the precursor cells that give rise to the brain. *Xenopus* tadpoles with the mutant gene developed neurological anomalies similar to those

[2] Seminara SB, Seminara MD, Messager S, Chatzidaki EE, Thresher RR, et al. The *GPR54* Gene as a Regulator of Puberty. *N Eng J Med* 349:1614–1627, 2003.

seen in RTT.[3] The researcher's findings contribute to an improved understanding of RTT, which may eventually lead to new treatments for the disorder.

*Gene discovered for Cornelia de Lange syndrome.* For the first time, scientists have found a mutated gene that is associated with Cornelia de Lange syndrome, a rare, multi-system disorder characterized by mental retardation, heart defects, and multiple other physical and behavioral anomalies.[4] Researchers studied 12 families in which one or more members have the disorder and identified a gene that had multiple mutations and was widely expressed in fetal and adult tissues. The gene appears to be involved in the very early stages of embryonic development and contains information needed to switch on a number of other genes during that period. The gene's discovery is expected to speed development of a prenatal test for the syndrome. A similar test will also be developed to diagnose Cornelia de Lange syndrome in young children who may have the condition. Discovery of the gene is an important step not only toward understanding and helping to diagnose the disorder but also for possibly developing future interventions to prevent it.

*Estrogen, genetic deficiencies, and cognitive function in Turner syndrome.* Girls and women with Turner syndrome (TS) are born with just one X chromosome in some or all of their cells instead of the two X chromosomes found in normal women. Approximately 60,000 girls and women are affected in the United States, with approximately 800 new cases diagnosed each year.[5] Individuals with TS lack normal estrogen production, are infertile, and may have coronary or other physical problems. Women and girls with TS usually have normal intelligence, but they may have specific cognitive deficits, including difficulty in learning math and performing visual-spatial coordination tasks such as mentally rotating objects in space. Although the evidence is mixed, some researchers have demonstrated positive effects of estrogen replacement on cognition and behavior in women who lack adequate levels of the hormone for various reasons. Whether this means that the lack of estrogen in TS explains the syndrome's cognitive deficits or whether the cause is lack of an X chromosome or a combination of hormonal and genetic factors is not known. Recently, NICHD scientists reported that the missing X chromosome appeared to be largely responsible for the distinctive cognitive profile of women and girls with TS.[6] Comprehensive cognitive evaluations of women with TS, women with premature ovarian failure (POF), and normal women indicated that the women with two X chromosomes—POF and normal—resembled each other and differed from the TS women in key cognitive tasks. The TS and POF women were treating their estrogen deficits with replacement therapy. Because they and the women without the conditions had comparable estrogen levels, researchers were able to attribute the cognitive deficits observed in the TS women to the missing chromosome.

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[3] Stancheva I, Collins AL, Van den Veyver IB, Zoghbi H, Meehan RR. A Mutant Form of MeCP2 Protein Associated with Human Rett Syndrome Cannot Be Displaced from Methylated DNA by Notch in *Xenopus* Embryos. *Mol Cell* 12: 425–435, 2003.

[4] Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, et al. Cornelia de Lange Syndrome Is Caused by Mutations in *NIPBL*, the Human Homolog of *Drosophila melanogaster Nipped-B*. *Nat Genet* 36:631–635, 2004.

[5] Turner Syndrome Society of the United States. Available at: http://www.turner-syndrome-us.org/resource/faq.html (cited December 2004).

[6] Ross JL, Stefanatos GA, Lushner H, Bondy C, Nelson L, et al. The effect of genetic differences and ovarian failure: Intact cognitive function in adult women with premature ovarian failure *versus* Turner Syndrome. 2004 *J Clin Endocrinol Metab* 89:1817–1822.

*Gender differences in Fragile X-related tremor/ataxia.* Fragile X syndrome is the most common form of mental retardation in boys and men and appears less frequently in girls and women. This developmental disorder is caused by a mutated gene in the X chromosome. A less than fully mutated or "permutation" form of the gene does not generally cause the more serious neurodevelopmental consequences of the full mutation but may cause a progressive neurological disorder in individuals as they age. It is estimated that approximately one in 3,500 to 8,900 males is affected by the full mutation and that 1 in 1,000 males has the premutation form of the gene.[7] Also, it is estimated that 1 in 250 to 500 females has the permutation and that 1 in 4,000 females is affected by the full mutation.[8] The first symptoms of this disorder, known as fragile x-related tremor/ataxia syndrome or FXTAS, are involuntary trembling (tremors) that make it difficult to write and perform other tasks or problems with balance (ataxia) and frequent falls. FXTAS progresses slowly to dementia and other more serious symptoms. Until recently, FXTAS was reported only in men, but NICHD-supported researchers have now reported the syndrome in a small number of women.[9] Unlike men, none of the women had dementia, suggesting that workups of families with the mutation or permutation should include neurological symptoms in older women as well as older men.

*Outcomes of treating girls for abnormal exposure to masculinizing hormones.* Girls with congenital adrenal hyperplasia (CAH) are genetically female but are exposed to abnormal levels of masculinizing hormones (androgens), beginning before birth. This condition affects about 1 in 10,000 to 18,000 children.[10] The external genitals of newborn CAH girls may be ambiguous or resemble those of boys. As these girls grow, their elevated androgen production affects their cognitive functioning, behaviors, physical growth, and gender identity. The standard medical treatment of female CAH consists of surgery, within weeks of birth, to correct genital anomalies, plus ongoing endocrine treatment and advice to parents to raise these "intersex" children as girls. Little systematically gathered information exists, however, on the long-term effects of these interventions, and there are questions about optimal treatment, including the timing of surgery, to ensure the best psychosexual outcomes. NICHD-supported researchers are now studying a group of CAH women to determine the degree of their androgen exposure at various developmental stages, to assess their psychosexual development, and to examine the factors, including the timing and type of surgery, that influence that development. This research is expected to improve clinical treatment of female CAH and, more generally, to enhance understanding of androgens' influence on individual differences in the development and expression of cognitive and sexual behavior.

*Possible behavioral effects of gene causing abnormally early puberty in boys.* A genetic defect that interferes with hormonal control of testosterone production in boys causes the rare condition familial male-limited precocious puberty (FMPP). Boys with FMPP can become sexually mature as young as age two. In addition to virilization and rapid physical growth and bone maturation,

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[7] Crawford, DC; Acuña, JM; and Sherman, SL (2001). FMR1 and the Fragile X Syndrome: Human Genome Epidemiology Review. *Genetics in Medicine* 3(5):359–371.
[8] Bailey, DB, and Nelson, D (1995). The Nature and Consequences of Fragile X Syndrome. *Mental Retardation and Developmental Disabilities Research Reviews* 1:238–244.
[9] Hageman RJ, Leavitt BR, Farzin F, Jacquemont CM, Greco JA, et al. Fragile-X-Associated Tremor/Ataxia Syndrome (FXTAS) in Females with the *FMR1* Premutation. *Am J Hum Genet* 74:1051–1056.
[10] Medline Plus. *Medical Encyclopedia*. Available at: http://www.nlm.nih.gov/medlineplus/ency/article/000411.htm (cited December 2003).

libido and aggressive behaviors are seen in young patients with FMPP. Until now, scientists have thought that these behaviors were secondary to FMPP—that is, that boys with FMPP experience behaviors seen in normal adolescent boys as they mature sexually. Recent studies suggest, however, that the same gene mutation that causes the early onset of puberty could also act in the brain to produce the abnormal behaviors seen in FMPP. To test this hypothesis, NICHD scientists are creating an experimental mouse with the same genetic defect and are using the animal model to study the location and distribution of the key hormone in the brain and the behaviors of mice with and without the mutation. While current treatments normalize rates of growth and improve other signs and symptoms of FMPP, understanding the causes of abnormal feelings and behaviors seen in these young boys would help scientists design better treatments for, and improve the management of, this disorder.

**Significant Ongoing Rare Diseases Research Initiatives**

**Mental Retardation and Developmental Disabilities Research Centers (RFA-04-024).** The NICHD continues to support the Mental Retardation and Developmental Disabilities Research Centers program to advance diagnosis, prevention, treatment, and amelioration of mental retardation and developmental disabilities. The purpose of the program is to provide core support and facilities for cohesive, interdisciplinary research and research training. Research projects may include genetic, molecular, and behavioral research to develop new treatment approaches for rare conditions or disorders.

**Fragile X Syndrome Research Centers (RFA-HD-02-009).** In response to Congressional priorities, the NICHD has created three new research centers for studies of Fragile X syndrome, the most common form of inherited mental retardation. To ensure the most productive research collaborations and to maximize efficient use of investigator expertise, study protocols, data collection and analysis, and other scientific resources, the NICHD established the new Fragile X sites as "centers within centers" in the Institute's longstanding Mental Retardation and Developmental Disabilities Centers. Scientists leading the new centers report that this innovative research model is substantially increasing scientific collaborations, attracting new researchers to the field, and resulting in productive expansion of our research efforts in Fragile X.

**New/Planned Extramural or Intramural Research Initiatives**

**Comparative Genetics of Structural Birth Defects (RFA-HD-03-024***).* Birth defects are the leading cause of infant mortality in the United States. Factors that change normal infant growth or development result in physical and functional defects. Physical or structure birth defects include cleft palate, heart defects, and neural tube defects. Spina bifida, one of the most severe structural birth defects, affects 1,500 to 2,000 babies in the United States each year.[11] Scientists use animal models to better understand the causes of such outcomes; however, few scientists study structural birth defects in more than one animal model. Understanding the development of multiple animal models may speed our understanding of the genetic causes of structural birth defects. To stimulate collaborations among scientists who study different animal models, the NICHD developed a new

[11] March of Dimes. Quick Reference and Fact Sheets. Available at: http://www.modimes.org/professionals/681_1224.asp (cited December 2004).

program where scientists are comparing genes, gene products, or pathways known to be important in the development of one animal model to another, less-characterized model. New research projects include examining molecular mechanisms linked to congenital skeletal disorders and other basic mechanisms linked to normal embryonic development.

**Rare Disease-specific Scientific Conferences, Symposia, or Workshops**

The NICHD cosponsored several meetings, conferences, and symposia with the NIH Office of Rare Diseases (ORD).

**Signaling in Vertebrate Organogenesis (February 26 – March 2, 2004).** One of the most challenging problems in human health is to understand what controls organ development and how this development can go awry to give rise to congenital malformations. To address this issue, the NICHD, with ORD support, held a conference to discuss how molecular mechanisms influence vertebrate organogenesis and how abnormalities in developmental processes can lead to structural birth defects and disease.

**What Have We Learned about Collaborative Clinical Investigation and Trials for Rare Genetic Diseases? What Works, What Doesn't, and Why? (March 4, 2004).** The NICHD, with support from the ORD, held a meeting to organize and operate a series of future workshops to evaluate the needs and opportunities for collaborative research on the prevention, intervention, and epidemiology of rare genetic diseases. Participants discussed the need for a large national level collaborative study mechanism to facilitate research and development for thousands of rare/orphan and ultra-rare genetic diseases.

**Rare Illnesses in Childhood: Emergency and Critical Care Presentation and Management to Maximize Outcomes (June 6–7, 2004).** With ORD support, the NICHD held a symposium to highlight the current state-of-the-art care that minimizes disability and improves outcomes for critically ill children with rare diseases, rare disorders, and injuries. Presentations included care for cardiac failure, respiratory failure, status epilepticus, hepatic and renal failure, overwhelming acidosis and metabolic errors, and brain injury/coma.

**The World Congress on Chromosome Abnormalities (June 27–30, 2004).** The ORD cofunded a meeting that the Institute held to discuss how specific treatments could be optimized for children born with chromosome abnormalities. Participants discussed the consequences of chromosomal abnormalities on specific organs and tissues. With this knowledge, researchers may be better able to devise treatment regimes specific to a chromosomal deletion or duplication. The overall goal is to help children with chromosome abnormalities to lead healthier and more independent lives.

**At the Crossroads: Common Pathways in Fragile X and Autism (July 7–8, 2004).** In collaboration with the ORD, the NICHD held a workshop that brought together leaders in the fields of autism spectrum disorders and Fragile X to examine future research directions to accelerate a comprehensive understanding of these disorders. Participants examined the basic science, clinical, and epidemiological evidence on the Fragile X permutation and its effects on reproduction. In addition, the meeting served to stimulate collaborative multidisciplinary research to examine the effects of fragile X permutation on premature ovarian failure.

**Fetal Therapy: Needs Assessment and Future Directions (August 16–17, 2004**). The NICHD, with ORD support, held a conference to stimulate researchers in a variety of fields, including fetal surgery, obstetrics, neonatology, maternal-fetal research, and pediatric surgery, to develop a plan to evaluate and disseminate fetal surgical innovations and to further the scientific evaluation of fetal surgery. The conference involved all groups that are practicing fetal surgery as well as specialists from other disciplines such as geneticists, genetic counselors, developmental pediatricians, ethicists, and statisticians, among others.

**Pineal Cell Biology Gordon Research Conference (August 29–September 3, 2004).** With cofunding from the ORD, the Institute held a meeting to discuss recent advances in circadian clock mechanisms controlling the pineal gland. Participants discussed the interactions of photoreception and metabolites, signal transduction, temporal gene expression patterns, and effects of melatonin on physiology and circadian biology.

## NATIONAL INSTITUTE ON DEAFNESS AND OTHER COMMUNICATION DISORDERS (NIDCD)

### Overview of Rare Diseases Research Activities

NIDCD conducts and supports research and research training on normal mechanisms and diseases and disorders of hearing, balance, smell, taste, voice, speech, and language. This mission is achieved through a wide range of research performed in its own laboratories, a program of research grants, individual and institutional research training awards, career development awards, center grants, cooperative clinical trials, and contracts to public and private research institutions and organizations. The Institute also conducts and supports research and research training that is related to disease prevention and health promotion. NIDCD addresses special biomedical and behavioral problems associated with individuals who have communication impairments or disorders. NIDCD also supports efforts to create devices that substitute for lost and impaired sensory and communication functions.

### Recent Scientific Advances in Rare Diseases Research

*Mitochondrial Genes and Deafness*

Mitochondria are specialized structures within cells that play a crucial role in metabolism and energy production. Mitochondria contain their own genes, which replicate during cell division. All of the mitochondria present in individuals are derived from the mother's egg. Therefore, diseases that appear to be passed exclusively through the maternal lineage are often linked to defective mitochondrial genes.

NIDCD-supported scientists have identified several specific mitochondrial mutations that predispose an individual to hearing damage resulting from toxicity from the aminoglycoside class of antibiotics to the inner ear hair cells. These investigators have determined that genetic loci in the nucleus of the cell act to modify the effects of the mitochondrial mutations. Most recently, a specific gene was identified in mice that modulates the severity of mitochondrial deafness and is also implicated in age-related hearing loss. This mouse model will be extremely valuable for detailed studies of the molecular mechanisms by which mitochondrial mutations contribute to deafness. These findings could be used to develop genetic tests to determine whether an individual has an increased risk for aminoglycoside-induced hearing damage.

*Usher Syndrome*

Usher syndrome (USH) is characterized by hearing loss and retinitis pigmentosa (RP). About 5 percent of individuals who are deaf have USH, and more than half of the deaf and blind individuals (>10,000) in the United States have USH. The severity of the hearing loss and the presence of vestibular dysfunction distinguish two major clinical subtypes of USH, types 1 and 2. Individuals who have USH type 1 are congenitally deaf and have a balance deficiency at birth, while RP has an onset at about the time of puberty. Individuals with USH type 2 are distinguished from USH type 1 in having a less severe hearing loss. A third form of USH (type 3) is characterized by progressive loss of hearing and retinal function. There are more than 11 different

genes in which mutations can cause USH. NIDCD intramural scientists have identified and characterized some of the genes responsible for USH and two common mutations that cause USH in the Ashkenazi Jewish population. They have discovered that the genes for USH type 1D and type 1F both encode cell adhesion proteins cadherin 23 and protocadherin 15, respectively. In addition, several NIDCD-supported scientists reported cloning the gene for USH type 2A. The *USH2A* gene encodes a protein, Usherin, that has structures similar to other proteins involved in assembling cells and tissues into functional organs. NIDCD-supported scientists also have identified the genes responsible for Usher type 1C. These advances are critical steps toward developing strategies to treat this devastating disease that causes deafness and blindness.

*Waardenburg Syndrome*

Waardenburg syndrome (WS) is an autosomal-dominant disorder that is characterized by pigmentary disturbances and deafness. NIDCD-supported scientists are seeking to determine the loci for WS type 2 by utilizing a high-density genome scan coupled with linkage analysis to identify candidate gene mutations that could be the cause of this disorder in three large, multigenerational families and several smaller families with WS2. Other scientists are studying the Dalmatian as an animal model for understanding the genetics of pigment-associated deafness in the dog and human. The relationship between pigmentation and deafness is not unique in Dalmatians and this model offers a unique opportunity to conduct genetic analysis of hereditary deafness.

*Auditory Neuropathy*

A small but substantial number of individuals with bilateral hearing loss have normal cochlear function. These individuals have severely abnormal central neural processing of auditor sensory input as evidenced by poor or absent auditory brainstem responses. Standard treatment strategies for bilateral hearing loss, such as hearing aids, are of little use to these individuals. When this disorder strikes young children or infants, it can cause severe disruption of normal language and speech development. The most likely cause of hearing loss is a disorder of the auditory nerve, hence the term “auditory neuropathy.” This disorder is rare but more common than previously expected. Investigation of the physiologic mechanisms, the genetic basis, and possible treatments for this disorder is ongoing.

*Endolymphatic Sac Tumors in von Hippel-Lindau Disease*

NIDCD intramural scientists are studying individuals affected by von Hippel-Lindau (VHL) disease and tumors of the inner ear. These endolymphatic sac tumors (ELSTs) have been found to develop in approximately 10 percent of individuals carrying mutations of the *VHL* gene. Hearing loss, balance disturbances, and tinnitus represent the primary clinical manifestations of this disease. Recent molecular genetic studies have confirmed the phenotypic association of ELST with VHL disease by demonstrating loss of heterozygosity at the *VHL* locus in tumor cells obtained from surgical specimens. In a clinical trial to preserve hearing in individuals with early stage ELST, preliminary results have revealed that these tumors can be safely removed while preserving hearing at preoperative levels and maintaining or improving vestibular function.

Prospective studies of this population of individuals should provide insight into the natural history of hearing and balance disturbances associated with ELST, while basic investigations will focus on the mechanisms by which ELSTs cause dysfunction of hearing and balance.

*Enlarged Vestibular Aqueduct*

Enlarged vestibular aqueduct (EVA) is characterized by progressive childhood sensorineural hearing loss in association with enlarged vestibular aqueducts. Recent data indicate that at least some cases are associated with mutations in the Pendred syndrome gene (PDS). Individuals with Pendred syndrome have sensorineural deafness and goiter. NIDCD intramural scientists are working to identify the genetic basis of EVA, including several cases where it is clearly not caused by mutations in PDS. In addition, the role of congenital cytomegalovirus infection in this form of hearing loss is also being studied.

*Stickler Syndrome*

Stickler syndrome is a genetic disease affecting the connective tissues of organs throughout the body. Stickler syndrome can affect the inner ears, resulting in permanent sensorineural hearing loss. Studies on inner ears of normal mice as well as a mouse model for Stickler syndrome (the *chondrodysplasia* mouse) have been completed by NIDCD intramural scientists and reveal how and where mutations in fibrillar collagen genes cause hearing loss in Stickler syndrome. The mutations act to disrupt the functions of normal genes and their corresponding protein products in the tectorial or basilar membranes of the cochlea, where these genes are specifically expressed. This disruption of gene function likely causes hearing loss by disrupting the biomechanical properties of sound wave propagation within the cochlea.

*Nonsyndromic Deafness*

Isolated deafness affects approximately 1 in 1,000 newborns and infants. Mutations in any one of nearly 100 genes can cause childhood deafness. NIDCD intramural scientists have recently identified several novel deafness genes through genetic mapping studies of large families with hereditary deafness from Pakistan and India. Identification of these genes increases our ability to diagnose hereditary deafness with molecular tests, and studies of their function in normal and pathologic states provide fundamental insights into normal hearing and the pathogenesis of hearing loss.

*Pendred Syndrome*

Pendred syndrome is a genetic disorder causing deafness in combination with, in some cases, enlargement (goiter) of the thyroid gland. Pendred syndrome is caused by mutations in the *SLC26A4* gene. NIDCD intramural scientists have studied the genetic epidemiology of deafness (and thus Pendred syndrome) caused by *SLC26A4* mutations in east and south Asia, which contain approximately one-half of the global population. In some populations, such as Koreans, *SLC26A4* mutations are the most common known cause of deafness. In all studied populations, *SLC26A4* mutations account for approximately 10 percent of all genetic deafness in childhood. This is a significant proportion, given that there are dozens of genes in which mutations can cause genetic

deafness. This study also demonstrated that ethnic groups each have their own distinctive spectrum of mutations, with one or a few most prevalent mutations. These results have significant implications for the design and implementation of molecular genetic tests for childhood deafness.

*Resistance to Thyroid Hormone*

Resistance to thyroid hormone (RTH) is a genetic disease causing resistance of target tissues to the actions of circulating thyroid hormone. RTH is caused by dominant mutations in the gene encoding the thyroid hormone receptor beta subunit. Intramural scientists from NIDCD and the National Institute of Diabetes and Digestive and Kidney Diseases have demonstrated that some individuals with RTH develop permanent sensorineural hearing loss. A mouse model for RTH was generated at the NCI and used for studies of auditory function and structure by NIDCD scientists. The results indicate that RTH mutations cause hearing loss through dominant-negative effects upon one or more other genes. Other studies by NIDCD-sponsored scientists revealed that this other affected gene likely encodes the thyroid hormone receptor alpha subunit. The disruption of the functions of the thyroid hormone receptor alpha and beta genes by RTH mutations results in hearing loss due to retarded development of the neurosensory tissues of the inner ear, which are dependent upon thyroid hormone for normal development and function.

*Acoustic Neuroma*

Neurofibromatosis type 2 (NF2) is an autosomal-dominant disorder that occurs in about 1 out of every 40,000 Americans. This mutation on chromosome 22 is strongly associated with the development of bilateral vestibular schwannomas, which then results in damage to both auditory nerves. Treatment of these acoustic neuromas often requires bilateral removal of the auditory nerves, which usually renders the individual deaf. Electrical stimulation of the residual neural pathways within the cochlear nucleus can provide a sense of hearing after this surgery. NIDCD-supported scientists are working to optimize the design of a neural implant used for electrical stimulation of the cochlear nucleus in these individuals, with the goal of providing a device equal in performance to the cochlear implant used in individuals with profound hearing loss.

*Hereditary Cerebellar Ataxia Syndrome of Early Onset*

Several abnormal genes that are associated with inherited cerebellar syndromes that cause disorders of balance and coordination have been identified. Relatively little is known about how different mutations lead to specific types of the disorder. There are typically great differences in the clinical signs and symptoms within families that segregate the same mutation and across families with mutations in the same gene. NIDCD-supported scientists have previously demonstrated linkage to chromosome 19p in four families with episodic vertigo and the inability to coordinate muscle movement (ataxia). The scientists have identified a missense mutation in the calcium channel gene on chromosome 19p in a family with severe progressive cerebellar ataxia of early onset involving the trunk, the limbs, and speech function.

*Olfactory Function*

NIDCD-supported scientists are investigating relationships between decreased olfactory function and a number of rare diseases. Studies have shown that olfactory loss appears to be among the first signs of such common neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Recent psychophysical studies have evaluated the prevalence and magnitude of olfactory loss in subtypes of Parkinson's disease, Down syndrome, schizophrenia, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and the rare amyotrophic lateral sclerosis/Parkinsonism/dementia complex of Guam. Better understanding of the associations between olfactory function and rare diseases may lead to earlier diagnosis and improvement in monitoring of these rare diseases.

*Kallmann Syndrome*

Kallmann syndrome is a rare genetic disorder with two main symptoms: an absence of the ability to smell and failure of the gonads to mature. There is a five- to seven-fold chance that this syndrome occurs in males in comparison to females, suggesting that the X-linked form of the disease is the most frequent. NIDCD-supported research has led to the identification of a common developmental defect in nerve migration, which links the two major disease symptoms. A unique family of proteins and their receptors that regulate nerve migration and direction during development are under investigation by NIDCD-supported scientists. Additional research is focused on isolating and cloning an X-linked gene responsible for Kallmann syndrome.

*Carcinoma of the Vocal Tract*

Cancers that occur in the mouth, throat, and vocal tract are less common than breast, prostate, or lung cancer but have a significant impact on voice, speech, and swallowing. There are approximately 40,000 new cases and 12,000 deaths each year and more than 320,000 survivors of head and neck cancer living in the United States. Approximately 80 percent of these cancers occur in persons who use tobacco and alcohol. A subset of tumors occurring in the tonsillar and adenoid areas have been associated with human papilloma viruses that also cause cervical cancer and Epstein-Barr virus that also causes mononucleosis. There is also an increased risk of this type of cancer in persons with Fanconi's anemia, a rare inherited disorder in which there is increased susceptibility to DNA damage, anemia, and cancer. NIDCD intramural scientists are collaborating with molecular biologists and clinicians from the National Cancer Institute to address the molecular basis for the disease and possible new treatments. These tumors show an increased response to growth factors when compared to other cells. Specific intracellular signaling molecules that mediate this effect have been identified. In addition, these tumors produce factors that stimulate the blood supply and immune cells in ways that help promote tumor growth and spread. Drugs that block the effects of these signaling pathways and factors may provide new approaches for prevention and therapy of these cancers.

*Velo-Cardio-Facial Syndrome/Di George Syndrome*

Velo-cardio-facial syndrome (VCFS) is a disorder that has been associated with more 30 different features, the most common being cleft palate, heart defects, characteristic facial features, minor

learning problems, and speech and feeding problems. VCFS is also known as Shprintzen, DiGeorge, cardiofacial, or conotruncal anomaly unusual face syndrome. These syndromes result from a large deletion at chromosome 22q11. VCFS is inherited in only about 10 percent to 15 percent of cases; however, in most instances, neither parent has the syndrome or carries the defective gene, and the cause of the deletion in the affected child is unknown. NIDCD-supported scientists have completed a detailed sequence analysis of the DiGeorge chromosomal region (DGCR) of chromosome 22q11. The 22q11.2 deletion occurs more frequently than originally anticipated, and the end points of the deletions occur in clusters. There is considerable variability in the abnormalities associated with deletions of similar size. The presence of a deletion is not always sufficient to cause cleft palate, strongly suggesting that modifier genes interact with the genes of the deletion region. Recent research has shown that the Clathrin heavy chain-like gene is a strong candidate gene for VCFS.

**Rare Disease-specific Scientific Conferences, Symposia, and Meetings**

On June 16–17, 2003, the NIDCD conducted a workshop on *"Neurological Motor Speech Disorders in Adults: Research Needs and Opportunities,"* to discuss neuroimaging in speech production disorders, central neural control of speech production, and how speech can be better assessed in neurological disease. The NIH Office of Rare Diseases cosponsored the workshop.

## NATIONAL INSTITUTE OF DENTAL AND CRANIOFACIAL RESEARCH (NIDCR)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Dental and Craniofacial Research is to improve oral, dental, and craniofacial health through research, research training, and the dissemination of health information. NIDCR's programs encompass basic, translational, and clinical studies of the broad range of diseases, disorders, conditions, and syndromes involving the oral cavity and craniofacial structures. NIDCR's section of this report highlights selected scientific advances within the Institute's intramural and extramural programs and other related program activities relevant to rare diseases that fall within NIDCR's mission.

### Recent Scientific Advances in Rare Diseases Research:

*Alzheimer's Disease and Amyotrophic Lateral Sclerosis*

Alzheimer's disease and amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases characterized by selective loss of motor neurons in the brain and spinal cord. Dysregulation of an enzyme known as cyclin-dependent kinase 5 (Cdk5) expression has been implicated as playing a critical role in processes underlying neurodegenerative diseases. In order to analyze the role of Cdk5 in such disorders, NIDCR scientists have developed many mouse models in which Cdk5 expression is genetically altered in one or more areas of the brain, during or after embryonic development. In one instance, studies of Cdk5-null mice revealed a neurodegenerative process typically seen in ALS. In another, specific elimination of Cdk5 in the frontal brain cortex resulted in drastic neurodegeneration similar to that observed in patients with advanced Alzheimer's disease. Current studies are focused on further delineating the roles played by Cdk5.

*Amelogenesis and Dentinogenesis Imperfecta*

Amelogenesis imperfecta (AI) and dentinogenesis imperfecta (DI) are the most common hereditary diseases affecting tooth enamel and dentin, respectively.

- AI is a heterogeneous group of rare inherited disorders that affect the formation of dental enamel and result in thin, malformed enamel that is easily abraded. As a tooth develops from its bud, an organic matrix forms that gradually crystallizes into tooth enamel. This process destroys nearly all traces of the matrix, along with clues to key information in understanding tooth formation. A team of NIDCR scientists report for the first time that the proteins amelogenin and ameloblastin interact in the matrix before mineralization proceeds. Understanding molecular interactions in the developing organic matrix of the tooth is necessary for understanding the causes of mineralization disorders such as AI and DI.
- Mutations of enamelin and amelogenin are known to cause AI. Studies of human families have recently resulted in identification of additional genes (known as the kallikrein gene and the MMP20 gene) that, when mutated, cause AI. In addition, studies of human families with AI have shown that some enamelin gene mutations cause only enamel pitting when present as a single copy, while the same mutation present in two doses results in severe enamel defects and

altered craniofacial structure, resulting in malocclusion. This is the first evidence for a gene dosage effect for enamelin mutations, which has important implications for understanding the variable clinical findings often reported for AI. These findings also suggest that the clinical phenotype of AI may need to be expanded beyond a simple tooth-related phenotype.

- NIDCR scientists recently created mice deficient in the enamel matrix protein ameloblastin. The mutant mouse is characterized by severe enamel hypoplasia, a disease similar to AI in humans. In the mutant mice, ameloblasts, cells responsible for production of enamel matrix proteins, continue to proliferate. A significant number of mutant mice develop oral tumors in the maxilla with age. These tumors are likely derived from mutant ameloblasts defective in ameloblastin. Deficiency of ameloblastin is also likely one of the causes of ameloblastoma, the most common human odontogenic tumor.
- DI is an inherited disorder that primarily affects dentin mineralization. It is classified into three subtypes: type I is the least severe, and type III is the most severe. Type I is also associated with osteogenesis imperfecta, while the more severe forms are restricted to dentin. Several mutations in the dentin sialophosphoprotein (DSPP) gene were recently identified in families with the type II disorder. In collaboration with extramural researchers, NIDCR scientists generated DSPP-deficient mice to characterize molecular roles of DSPP in tooth development. These mice develop tooth defects similar to DI in humans. NIDCR researchers now are analyzing the cooperation between DSPP and genes expressed in hard tissues. Along with NICHD investigators, they also have initiated an analysis of tooth defects in a mouse model for osteogenesis imperfecta.

*Behçet's disease*

Behçet's disease is a chronic condition that causes severe oral ulcers and ulcerations in other mucosal sites. The exact cause of Behçet's disease is unknown and currently there is no effective therapy. NIDCR scientists are using salivary gland gene transfer to produce proteins that will augment saliva and potentially promote healing of the oral ulcerations in Behçet's patients.

*Dentin Dysplasia*

Dentin dysplasia is a rare hereditary disorder resulting in dentin defects such as thin and broken dentin, resulting in chipped teeth susceptible to infection. In order to analyze the involvement of growth factors in tooth development, NIDCR scientists developed transgenic mice in which a growth factor (TGF-beta1) is overexpressed. Unexpectedly, these mice developed distinct tooth defects similar to those seen in dentin dysplasia. Currently, NIDCR researchers are characterizing multiple roles of the TGF-beta signaling pathway in tooth biology.

*Cleft Lip/Cleft Palate*

- Scientists have gained new insight into the mechanism underlying cleft lip/cleft palate (CL/P) pathogenesis through the genetic mapping of Dancer mutant mice. Mice carrying a spontaneous mutation, Dancer (Dc), exhibit significantly increased susceptibility to CL/P. Researchers genetically mapped most of Dc chromosome 19. Analysis showed that one positional candidate gene, Tbx10, was ectopically expressed in Dc-mutant embryos;

furthermore, they showed that ectopic expression of Tbx10 in transgenic mice can be passed to the next generation. Animal models are a valuable source of information in understanding the complex genetic mechanisms underlying CL/P disorders.

- Researchers assessed the overall and cause of mortality of people from birth to 55 years with CL/P. This study used data from Danish registers to assess the long-term prognosis associated with CL/P, particularly overall mortality and cause-specific mortality. Investigators followed 5,331 people with CL/P. The expected number of deaths was 259, but 402 occurred. The increased risk of mortality was nearly constant for the three intervals at follow-up: first year of life, 1–17 years, and 18–55 years. The participants had an increased risk of all major causes of death. These findings suggest that children born with CL/P and possibly other congenital malformations may benefit from specific preventive health measures into and throughout adulthood.

*Coffin-Lowry Syndrome*

Coffin-Lowry syndrome (CLS) is an inherited, sex-linked disorder associated with craniofacial, dental, and skeletal abnormalities as well as mental retardation. It is caused by a mutation in RSK2, a gene that encodes a growth factor-regulated enzyme. Investigators supported by the NIDCR have accomplished the molecular dissection of an important signaling pathway and generated mouse models that will allow scientists to advance our understanding of this skeletal disorder and to design strategies for intervention. Specifically, they discovered that the transcription factor ATF4 is the substrate for RSK2 phosphorylation in osteoblasts. Therefore, mutation in RSK2 disrupts the signaling pathway to ATF4, which results in skeletal abnormalities seen in patients with CLS.

*Craniosynostosis*

Growth and expansion of the brain continues well into postnatal life and requires growth of the overlying calvarial bones of the skull. Premature fusion of the cranial sutures, or craniosynostosis, impedes this growth and results in an abnormal shape of the skull and defects such as blindness and mental retardation. Cranial neural crest (CNC) cells are multipotent cells that contribute extensively to head and neck structures including the palate and calvaria. Molecular mechanisms that regulate the fate of CNC cells during development are not well understood. Previous studies have shown that members of the transforming growth factor β (TGFβ) family play an important role in palatal fusion and skull development. A research team supported by NIDCR recently reported that loss of TGFβIIR results in a complete cleft secondary palate and defects in the calvaria, dura matter, and jaw. The current study is significant in that it provides new information about the role of CNC-derived mesenchyme during development and the molecular mechanism and signaling pathways regulating CNC proliferation.

*Dyssegmental Dysplasia*

Dyssegmental dysplasia, Silverman-Handmaker type (DDSH) is a rare inherited skeletal disorder characterized by abnormally shaped vertebral bodies and short limbs. Individuals with DDSH also have a flat face, abnormally small jaws, cleft palate, and reduced joint mobility. Recently NIDCR

scientists identified mutations of the perlecan gene (*HSPG2*) in three patients with DDSH. Investigation of the mutations indicates that DDSH is caused by mutations of the perlecan gene similar to perlecan gene-knockout mice. These results indicate that perlecan is essential for cartilage development. NIDCR researchers are studying the role of perlecan in cartilage development by identifying more mutations of DDSH through the creation of additional animal models.

*Fabry Disease*

Fabry disease is a familial sex-liked disorder of lipid metabolism in which glycolipid accumulates in many tissues. Major disease manifestations include pain in the extremities; angiokeratomas; corneal dystrophy; oral and dental abnormalities; and vascular disease of the heart, kidney, and brain, leading to premature death. Currently, patients are treated by systemic injections with the recombinant enzyme alpha-galactosidase. NIDCR scientists are collaborating with colleagues in NINDS to develop a gene transfer strategy employing salivary glands to treat this disease. Toward that goal, they have reported the generation and characterization of AGA-deficient mice with notable similarities to human patients with Fabry disease. NIDCR scientists are constructing recombinant viral vectors to deliver the enzyme alpha-galactosidase via the salivary glands of the mouse model of Fabry disease, and studies are in progress to analyze abnormalities in teeth and salivary glands of Fabry mice.

*Giant-cell Arteritis*

Giant-cell arteritis is a chronic granulomatous vasculitic disease primarily affecting elder women. The typical presenting feature is a continuous, throbbing temporal headache. Other symptoms include pain during chewing, talking, or swallowing; ocular or orbital pain; transient loss of vision; blurred vision; or sudden, permanent blindness. Some patients respond to corticosteroid therapy while others do not; the reason for this difference is not known. NIDCR scientists analyzed vessel biopsy specimens from therapy-responding and nonresponding patients for genes that might account for this difference. They identified and validated several genes that were differentially expressed in the nonresponding patients. Their investigations identify CCL2 (MCP-1) as a relevant molecule in perpetuating inflammatory lesions in giant-cell arteritis and conclude that it may be a marker for the nonresponding patients and serve as a potential therapeutic target.

*Growth Hormone Deficiency (Adult)*

Growth hormone deficiency (GHD) is a disorder most commonly caused by frank pituitary disease, often the presence of nonfunctional pituitary adenomas, or as a result of surgery or radiotherapy for pituitary adenomas. NIDCR scientists, in collaboration with NICHD colleagues, continue to develop gene transfer strategies to treat adult GHD using salivary glands as the target tissue. Their recent focus has been on reengineering GH so that after gene transfer to a salivary gland, it will be efficiently secreted into the bloodstream. To enhance GH secretion into the bloodstream, they manipulate putative sorting signals in GH. Several GH mutations were tested and one biologically active mutant displayed a relative increase in proportion of GH secretion from rat salivary glands into the bloodstream. The results suggest that the final destination of a transgenic secretory protein may be controlled by reengineering sorting determinants.

*Kaposi's Sarcoma*

Kaposi's sarcoma (KS) is the most common cancer arising in HIV-infected patients and the most frequent oral neoplasm in immunosuppressed patients. KS has also emerged as one of the most prevalent cancers among children and adult men in the developing world. The Kaposi's sarcoma-associated herpesvirus (KSHV; HHV-8) has been recently identified as the infectious cause of Kaposi's sarcoma. Of interest, compelling evidence now supports a critical role for the oral cavity as the primary source of infectious HHV-8 in both immunocompetent and immunosuppressed patients. The sequencing of the full KSHV genome revealed a candidate gene, vGPCR, which promoted the development of visible dermal and internal vascular tumors that strikingly resemble human KS lesions. The results implicated vGPCR in both the initiation and promotion of Kaposi's sarcoma. Subsequent studies examining the mechanism by which vGPCR acts are pointing to downstream targets, which may represent essential mediators of vGPCR-induced neoplasia.

*McCune-Albright Syndrome and Fibrous Dysplasia of Bone*

The McCune-Albright syndrome is defined by the triad of fibrous dysplasia of bone (FD), café-au-lait skin spots, and endocrine gland hyperfunction. Some patients may have only a single focus of bone affected, and others may have severe disease affecting multiple endocrine glands and virtually the entire skeleton. NIDCR scientists have five clinical research protocols under way studying various aspects of the disease, ranging from a study of the natural history of the disease to treatment studies for the bone and endocrine disorders. The natural history study is producing a number of findings resulting in better understanding of disease progression and patient care management. Clinical drug trials aimed to improve care for patients with this rare disease are ongoing. These findings, and those to come, have implications for the long-term prognosis and clinical management of patients with these rare disorders.

*Mucolipidosis-IV*

Mucolipidosis type IV (ML-IV) is a rare inherited metabolic disorder that leads to abnormal accumulation of certain fatty substances and complex carbohydrates in the cells of many tissues. It is generally characterized by mental retardation, impaired coordination of muscular and mental activities, and corneal opacity. Currently, there is no animal model available to develop effective therapies to treat this fatal disorder, but a recent advance by NIH scientists may change that situation for the better. In collaboration with NINDS investigators, NIDCR scientists have modified the ML-IV locus in mouse embryonic stem cells and have generated founder mice. These mice will be characterized in detail to identify whether they mimic ML-IV disease.

*Schwartz-Jampel Syndrome*

Schwartz-Jampel syndrome (SJS) is a rare inherited skeletal dysplasia associated with myotonia (muscle spasms or temporary rigidity). This disorder is characterized by short stature, abnormal development of the cartilage and bone, and a characteristic face with a "fixed" facial expression, narrowness of the fissure between the eyelids, pursed lips, and sometimes low-set ears and myopia. Skeletal abnormalities include spinal curvature, flattened vertebral bodies with coronal clefts, and

joint contractures. NIDCR scientists and others have recently identified mutations of the perlecan gene (*HSPG2*) of patients with SJS. Based on clinical examinations, the SJS phenotype appears to encompass a wide spectrum of disorders. NIDCR researchers are now examining for possible mutations of the perlecan gene in other skeletal dysplasias that were previously identified as chondrodysplasia, such as micromelic chondrodysplasia and Burton's disease. They have created a mouse model to study the mechanism of SJS myotonia by genetic manipulation. This mouse model will facilitate the development of strategies and reagents for therapy.

*Squamous Cell Carcinomas of the Head and Neck*

Squamous cell carcinomas of the head and neck result in approximately 11,000 deaths per year in the United States The majority involve neoplastic lesions in the oral cavity, lip, and pharynx. Although the incidence is rare compared to other cancers, these cancers remain among the most fatal and morbid of cancers at any anatomic site.

- As part of NIDCR's research program aimed at identifying the nature of those genes expressed during oral cancer development, a genome-wide analysis of nasopharyngeal carcinoma was recently conducted. This analysis enabled the identification of genes differentially expressed in normal, dysplastic, and cancerous cell populations and identified numerous genes whose overexpression can help explain the aggressive clinical nature of this tumor type as well as a decrease in genes involved in programmed cell death and tumor suppression.
- The protein kinase Akt is a key regulator of normal and cancerous growth and cell fate decisions. Recently it was found that Akt activation correlates closely with the progression of squamous carcinomas in mice and that activation of Akt is a frequent event in human oral cancer. Evidence was obtained that the Akt signaling pathway may represent a biologically relevant target for the novel chemotherapeutic agent UCN-01 at concentrations safely achievable in clinically relevant situations.
- Using transgenic mice, researchers have created an animal model that can promote the rapid (10–20 days) formation of malignant squamous carcinomas in the skin and oral tissues of animals treated with doxycycline. Current and future use of this animal model system may help to unravel the mechanisms responsible for squamous carcinomas and aid in the search for alternative oral cancer treatments.
- Epstein-Barr virus (EBV) is the causative agent for several human ailments, including oral hairy leukoplakia, infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal carcinoma. EBV infection of B lymphocytes is essential for infection in humans; however, the role of epithelial cell infection in the normal EBV life cycle remains controversial. Using an EBV-related herpesvirus that naturally infects rhesus macaques, researchers have shown that the virus can infect epithelial cells in immunosuppressed macaques and can induce epithelial cell lesions resembling oral hairy leukoplakia. These studies demonstrate a useful animal model for study of the pathogenesis of EBV infection in immunosuppressed hosts in which to perform controlled studies that are impossible to conduct in humans.
- One of the most persistent problems in the treatment of head and neck cancers is the high incidence of local and regional recurrence, due partly to lack of sensitive techniques that can detect minimal residual disease after surgery or monitor patients for cancer recurrence. NIDCR-funded investigators developed a molecular technique named Gap Ligase Chain

Reaction Assay that detects rare amounts of tumor/mutated DNA within a sample of primarily normal DNA. However, the application to clinical screening was hampered by the extended time required for preparation and analysis of the sample. Now they have described a modification termed Fluorescence Gap Ligase Chain Reaction. This assay can be performed in less than 5 hours, while the patient is undergoing surgery, providing crucial information on the spread and recurrence of head and neck tumors. The assay also can aid in molecular staging of head and neck cancers based on analysis of lymph nodes and/or serum.

- Scientists discovered several years ago that interleukin-12 (IL-12), a protein secreted by immune cells, can alert disease-fighting T cells to recognize, attack, and remember tumor cells for months to come. However, they have found the protein or its gene is most effective when injected directly into tumors, not infused into the bloodstream. In a key first step in this direction, a team of scientists has identified four genes in oral squamous cell carcinoma cells that respond particularly well to direct administration of IL-12. Two of the genes—IRF7 and Wsb2—are little studied by cancer researchers and could provide excellent targets for further investigation. The Il-12 gene was delivered directly into squamous cell carcinoma cells with a technique called electroporation, in which pulses of electricity are used to open up small channels into the cell through which Il-12 can be delivered. Electroporation could have an important future role in treating oral cancer, particularly when combined with other therapies. The work was supported jointly by the NIDCR and the NCI.
- Previous research has identified a link between the presence of human papillomavirus (HPV) and the risk for developing head and neck cancer. This finding opens a possibility of using HPV as an independent predictor of risk for this cancer. NIDCR-supported scientists used an oral rinse to recover cells that normally are shed in the mouth. They looked for the presence of high-risk HPV in the cells of head and neck cancer patients and compared them with persons of similar age and gender without the disease. They found that HPV high-risk types can be detected from oral exfoliated cells from an oral rinse. The finding may lead to the development of a screening test to prevent oral cancer or to detect it in the earliest stages.

*Temporomandibular Muscle and Joint Disorders*

Temporomandibular muscle and joint disorders (TMJDs) are a group of conditions causing pain and dysfunction in the temporomandibular joint and surrounding muscles. While there are no firm data on how many people are affected by TMJDs, orofacial pain is a major cause of poor quality of life. The prevalence of TMJDs is higher in women than men. Several recent studies have contributed new knowledge concerning associations between fluctuating levels of reproductive hormones and variations in both clinical TMJD pain and symptoms and responsiveness to experimentally induced pain. A prospective study of pregnant women with diagnosed TMJDs assessed variations in reported TMJD pain throughout pregnancy. A second study linked cyclic changes in levels of endogenous reproductive hormones with clinical pain levels reported by female TMJD patients. Findings from the studies suggest that clinical TMJD pain in women is highest when estrogen levels are at their lowest but that rapid estrogen change can also be associated with higher reported clinical pain levels. Results in rodents in a controlled laboratory setting also suggest that the differences in pain sensitivity found in humans are a real biological effect of sex hormones and not due entirely to the influence of environmental factors.

**Rare Diseases Program Activities**

**Scientific Conferences**

- *WHO International Collaboration to Reduce the Health-care Burden of Craniofacial Anomalies:* For 5 years, NIDCR has supported a World Health Organization (WHO) global effort titled, "International Collaborative Research on Craniofacial Anomalies." The goal of the project is to reduce duplication of efforts and achieve broader coverage of priority research needs by bringing together international researchers through collaborative partnerships and to develop global consensus on craniofacial anomalies research directions and common research protocols. On December 2–4, 2004, participants met in Geneva to evaluate the project, identify next steps, and make recommendations for the future. Published reports from the project are available on NIDCR and WHO Web sites: http://www.who.int/genomics/anomalies/en/ and http://www2.nidcr.nih.gov/research/international/CraniofacialAnomalies/index.asp. The WHO site also includes public access to the research resources and registry databases.
- *Gordon Research Conference on Craniofacial Morphogenesis and Tissue Regeneration*: NIDCR and NICHD cosponsored the newly established Gordon Research Conference on Craniofacial Morphogenesis and Tissue Regeneration. The international meeting, held in Ventura, CA, in January 2004, provided a forum for the exchange of information about the latest progress in craniofacial research. The conference focused on early events in craniofacial development that underlie human craniofacial defects. Future meetings will take place every 2 years.
- *Temporomandibular Joint Disorder:* "The Third Scientific Meeting of the TMJ Association: Advancing Diagnostic Approaches for TMJ Diseases and Disorders" was held May 6–7, 2004, in Bethesda, MD. The meeting was stimulated by the critical need to establish improved research and diagnostic criteria for temporomandibular diseases and disorders. Clinicians and scientists interested in these conditions have been handicapped by an absence of standardized, accepted diagnostic criteria. The goal of the organizers was to bring together experts from many fields to assess and explore novel approaches to current challenges. The meeting was cosponsored by NIDCR, NIBIB, NIAMS, Office of Rare Diseases, and the Office of Research on Women's Health.

**Awards**

- *Craniosynostosis:* NIDCR has funded a multi-site, 5-year longitudinal study in which infants with one of four types of single-suture craniosynostosis will be recruited: sagittal, metopic, right unilateral coronal, and left unilateral coronal. A case-matched "control" group of healthy, normal infants will also be followed. The long-term objectives are to chart the neurobehavioral course of single-suture fusions and to better understand how the developing cranium affects human brain growth and function. This research will take place in medical centers located in Seattle, Chicago, St. Louis, and Atlanta. Seattle's Children's Hospital and Regional Medical Center is the lead agency.

- *Squamous Cell Carcinoma of the Head and Neck:* NIDCR recently funded three projects to develop state models for oral cancer prevention and early detection. The projects, located in Michigan, North Carolina, and Florida, use various methods to increase screening and detection of oral cancers while decreasing known risk factors.

**Planned Activities**

- *Research Conference: Sjögren's Syndrome: Transition from Autoimmunity to Lymphoma:* An international conference organized by the Sjögren's Syndrome Foundation to explore the transition from Sjögren's syndrome to lymphoma will occur in Baltimore, MD, in September 2005. Lymphoma is approximately 40-fold more prevalent in Sjögren's syndrome patients than the general population, affecting 5–10 percent of patients. The conference will provide a forum for exchange of research findings by new and established investigators and provide opportunities to initiate or strengthen collaborations.

## NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES (NIDDK)

### Overview of Rare Diseases Research Activities

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) supports basic and clinical research on many rare diseases. Although diseases such as type 1 diabetes, type 2 diabetes, and obesity are not rare, there are rare single gene defects that cause these conditions such as Maturity Onset Diabetes of the Young (MODY) and lipodystrophy. In addition, the NIDDK supports research on both common and rare causes of kidney, liver, and GI diseases. The Institute also supports research on genetic metabolic diseases such as cystic fibrosis, lysosomal storage diseases including Krabbe disease and Mucopolysaccharidoses, disorders of amino acid metabolism such as tyrosinemia and maple syrup urine disease, disorders of copper transport including Menkes and Wilson disease, and hematologic diseases such as Cooley's anemia and sickle cell disease.

### Recent Scientific Advances in Rare Diseases Research

*Cystic Fibrosis*

Cystic fibrosis (CF) is the most common fatal genetic disease in Caucasians, affecting approximately one in 3,000 newborns. Patients are diagnosed in early childhood with symptoms such as failure to thrive. With management of the nutritional problems and infections, the life expectancy for CF has been increased to over 30 years. CF is caused by mutations in the gene encoding the CFTR protein, which resides in the outer surface of cells lining such tissues as the lung and intestine, where it regulates the movement of chloride. The most common mutation of the gene, ΔF508, yields a protein that does not fold properly and is thus degraded before it reaches the cell surface. Researchers have recently tested the effect of a compound called curcumin, purified from the spice turmeric, in a mouse model of CF. When given to mice that are genetically engineered to have the ΔF508 mutation, curcumin treatment enabled the mutant form of the CFTR protein to function effectively, presumably by promoting the correct folding to allow it to reach its normal cellular destination. Indeed, when cells cultured from animals with the ΔF508 mutation were treated with curcumin, the protein was properly routed to the cell surface. Importantly, the amount of curcumin that achieved these promising results in mice is equivalent to a dose that has been well tolerated by humans in previous studies. Therefore, curcumin, which is already known to be safe in people, has the potential to be of value for patients with this devastating illness.

*Tyrosinemia*

New approaches for treating liver disease are emerging from laboratory studies of hereditary tyrosinemia type I—a rare inherited metabolic disorder associated with severe liver and kidney disease in infants and children. It is caused by a deficiency in an enzyme that breaks down the amino acid tyrosine, resulting in elevated tyrosine levels in the blood (tyrosinemia) and tissue damage. Although a drug for treating this disease was approved in 2002, its long-term efficacy is still in question, and liver transplantation remains the most effective therapy. One way to correct the underlying defect in genetic diseases such as hereditary tyrosinemia would be through transplantation of cells with a

functioning copy of the gene for the missing enzyme. In recent years, researchers have explored this possibility in mice that are deficient in the same enzyme that causes hereditary tyrosinemia type I in humans. They found that transplantation of stem cells derived from the bone marrow of healthy adult donor mice into the mice with tyrosinemia resulted in fusion between the healthy and diseased cells, correction of the genetic defect, and repair of the liver. Investigators then wanted to identify which cell type was responsible for this correction. Were stem cells, with their ability to turn into a variety of cell types, required, or could more mature cells—already committed to forming a particular cell type—also work to correct the defect? Researchers addressed this by conducting a series of transplantation experiments using several different types of donor mice that were genetically engineered to produce only certain types of cells that originate in the bone marrow. They found that it was possible to correct the defect in mice with tyrosinemia by using macrophages—a kind of immune cell that develops from cells that form in the bone marrow. These results support the theory that donor stem cells used in prior experiments probably differentiated into macrophages prior to fusing with the recipient's liver cells. Importantly for potential clinical applications, this study also suggests that, in contrast to bone marrow transplantation, treatment with macrophages could be a less invasive, more efficient type of cell transplantation procedure for genetic liver diseases such as hereditary tyrosinemia. A key benefit of macrophages or their immediate precursor cells is that they could be administered directly into the liver or bloodstream.

*Polycystic Kidney Disease*

Polycystic kidney disease (PKD) and other inherited cystic kidney diseases frequently cause kidney failure and death; some rare forms of PKD primarily affect children. There are no effective treatments. One characteristic common to several of these disorders is an elevated level of cyclic adenosine monophosphate (cAMP) in the kidneys. Within cells, cAMP transmits messages that affect their growth and function; abnormally high levels of cAMP in certain kidney cells are thought to contribute to cyst formation. Researchers treated animal models of the two predominant forms of human PKD and another cystic kidney disease using a chemical, OPC31260, which lowers cAMP production in the kidneys. The treatment halted disease progression, and in some cases resulted in improvement. OPC31260 and similar compounds are currently undergoing testing in human clinical trials for treatment of other diseases and so far appear to be safe. Thus, drugs of this class are promising candidates for phase I clinical trials to treat patients with PKD.

*Mucopolysaccharidosis*

Mucopolysaccharidoses (MPS) are a group of disorders that result from a defect in enzymes needed to degrade mucopolysaccharides. These undegraded molecules become trapped and accumulate in the lysosomes of the cell, causing cell death. The incidence of this group of diseases is about 1 in 22,500 births. Aldurazyme, an enzyme replacement therapy (ERT) for MPSI, which is given intravenously every week, was introduced last year. Replacement of a missing enzyme can result in an immune reaction to this protein, which may be foreign to some patients with null mutations. In order to prevent a strong antibody response to this protein, a tolerization regimen has been developed in the dog model of MPSI. This regimen consisted of a 60-day course of cyclosporine A and azathiprine with low-dose infusions of the enzyme. The dogs tolerated the enzyme for up to 6 months following the treatment. This promising approach, which may be applicable to other disorders using ERT, needs to be tested in humans.

*Krabbe Disease*

Krabbe disease, also called globoid cell leukodystrophy, is a genetic lysosomal storage disease that occurs in approximately 1 in 200,000 births. This disease usually manifests in the first year of life, resulting in neuronal degeneration and eventually death. However, impressive results have been reported in patients who receive a cord blood transplant prior to the initiation of symptoms. In order to identify at-risk infants prior to the appearance of symptoms, a high-throughput screening test is needed for Krabbe disease. NIDDK-supported investigators have developed a newborn screening test for Krabbe disease using tandem mass spectrometry that can be performed on a dried blood spot. This technology paves the way for including newborn screening for Krabbe disease in newborn screening programs to allow early diagnosis so that a life-saving cord blood transplantation can be performed.

*Crohn's Disease*

Crohn's disease (CD) is a chronic, currently incurable digestive disease, most commonly affecting either the colon or the portion of the small intestine nearest to it, the ileum. Symptoms frequently include abdominal pain, nausea, vomiting, weight loss, and diarrhea, which is occasionally bloody. The precise causes of CD are unknown, but bacteria in the gut are thought to contribute. There may also be a genetic component: the disease not only runs in families but Americans and Europeans with the disease also frequently have particular variants of a gene called card15, which is expressed in immune cells and is believed to have a role in innate immunity to bacteria. In a new study, researchers found that the card15 gene is expressed at high levels in so-called Paneth cells, which lie at the base of invaginations in the small intestine. The Paneth cells secrete anti-microbial compounds, probably playing an important role in controlling gut bacteria. Thus, card15 and Paneth cells represent an apparent link between the genetic and bacterial risk factors for the illness and are a promising target for development of therapeutics.

*Hirschsprung Disease*

Hirschsprung disease (HSCR) is a multigenic, congenital disorder that affects 1 in 5,000 newborns and is characterized by absence of the enteric neurons in the distal gut. One of the primary genes involved in HSCR is the G protein-coupled endothelin receptor B gene (EDNRB). Expression of this gene is required for migration of the cells forming the enteric nervous system into the colon. To better understand the expression of this gene, NIDDK-funded investigators studied the regulatory region of this gene. They identified a region that modulates the expression of this gene through the binding of the neural crest transcription factor, SOX10. Engineered mice with a deletion or mutation of these sites did not express EDNRB and developed megacolon. These studies may explain the interaction among the genes that cause HSCR. In addition, understanding the function of this regulatory region could explain spatiotemporal regulation of gene expression.

**Rare Diseases Research Initiatives**

In September 2004, the NIDDK and NIH Office of Rare Diseases jointly funded the Rare Liver Diseases Consortium to expand studies in children with serious cholestatic liver diseases, including Alagille syndrome, progressive familial intrahepatic cholestasis, alpha-1-antitrypsin deficiency-

associated disease, the mitochondrial hepatopathies, and inborn errors of bile acid metabolism. These disorders have serious, if not fatal, consequences and severely affect normal growth and development. The Consortium will develop a longitudinal hypothesis-driven database study of these diseases and collect biosamples in the NIDDK Central Repository to facilitate future studies.

The NIDDK has undertaken a review of its Centers Program. The major recommendation was to encourage more translational research to be conducted in our disease-specific Centers. To accomplish this, NIDDK has put out RFAs for two new types of Centers: PKD Research and Translation Core Centers, DK-04-012, and CF Research and Translation Core Centers, DK-04-008. NIDDK expects to fund two new Centers from each RFA in 2005. In addition, the NIDDK reissued RFAs for two existing Centers programs that support rare disease research: Centers for PKD Research, DK-04-011, and Centers of Excellence in Molecular Hematology, DK-04-15. NIDDK expects to fund two PKD Centers and three Hematology Centers in 2005.

In partnership with NCI and NIAID, NIDDK issued PA-04-068, "Development of Assays for High Throughput Drug Screening." The purpose of this PA is to encourage the use of high-throughput small molecule screening in both research and drug discovery programs by funding the development of innovative assays that may be adapted for automated screening. The assays would aim to identify new tools for basic research and promising new avenues for therapeutics development. One specific bullet calls for research involving mutant proteins responsible for inborn errors of metabolism, cancer, or other rare diseases. Assays developed under this PA will be eligible for consideration by the NIH Molecular Library Screening Centers that are being developed as a component of the NIH Roadmap. The first applications submitted to this PA are expected to be funded in 2005.

**Rare Disease-specific Conferences**

On November 20–21, 2003, the NIDDK and the Oxalosis and Hyperoxaluria Foundation cosponsored a meeting on Oxalosis and Hyperoxaluria in Annapolis, MD. The chairs of the organizing Committee were Dawn Milliner, M.D., Mayo Clinic, and Gill Rumsby, Ph.D., University College London along with Craig Langman, MD, Children's Memorial Hospital Chicago on behalf of the Oxalosis and Hyperoxaluria Foundation. Marva Moxey-Mims, MD, and Rebekah S. Rasooly, Ph.D., of NIDDK served as co-organizers. A total of 23 lectures were given by senior and junior investigators in sessions titled Enzyme Form and Function, Oxalate and the Cell, Diagnosis of Primary Hyperoxaluria, and Toward Rational Therapeutics. In addition, a patient and the parents of a pediatric patient made presentations. Approximately 50 investigators, clinicians, and trainees from the United States, Canada, Europe, and Japan attended the workshop. The workshop featured much new research that was unpublished. Several especially noteworthy reports were of the promise of molecular chaperones for therapeutics by Jeff Kelly, Scripps, and Chris Danpure, University College London; and basic research and preliminary clinical studies using *Oxalobacter* as a treatment by Marguerite Hatch, University of Florida, and Bernd Hoppe, University Children's Hospital Cologne, Germany. In addition, Dawn Milliner, Mayo Clinic, and Gill Rumsby, University College London, presented unpublished data about the challenges of physiological and genetic diagnosis and led a discussion aimed at evolving a consensus algorithm for publication. Finally, John Lieske and Dawn Milliner of the Mayo Clinic presented the new

Web-based NIDDK-funded International Registry of Hereditary Calcium Urolithiasis. The meeting provided a much-needed forum for exchanging new research on primary hyperoxaluria and for developing a consensus among international experts about diagnosis and treatment of the disease. At the adjournment of the workshop, participants agreed to meet again in 2004 in Rochester, Minnesota.

On May 4 and 5, 2004, NIDDK, ORD and the Oxalosis and Hyperoxaluria Foundation sponsored a workshop titled, "Protein Misfolding and Misprocessing in Disease." Allen Spiegel (Director, NIDDK) and Steve Groft (Director, ORD) introduced the meeting, noting that the primary objective was to stimulate research that will translate basic cell biology, biochemistry, and biophysics findings about protein structure and assembly into potential therapies for monogenic and other misfolding and misprocessing disorders. In his introduction, Brett Rosen (Oxalosis and Hyperoxaluria Foundation) highlighted the human value of this research in providing hope for patients suffering from these diseases and for their families. The meeting was well attended, with 209 participants. The audience included clinicians, clinical researchers, and basic scientists at all career levels from academic institutions, the NIH, and the private sector. Twenty-one of the MDs who attended received CME credit. In all, there were 31 scientific talks, 4 of which were selected from abstracts submitted by junior investigators. Twenty-nine posters were presented at an active and crowded poster session. Rare diseases specifically covered in presentations: cystic fibrosis, nephrogenic diabetes insipidus; collegen diseases, including osteogenesis imperfecta; primary hyperoxaluria; Gitelman's syndrome; alpha-1-anti-trypsin deficiency; congenital hyperinsulinism; Wolfram syndrome; Gaucher disease; polycystic kidney disease; familial amyloidosis; familial amyloid polyneuropathy; congenital nephrotic syndrome; Fabry disease; GM1 gangliosidosis; and Tay-Sachs disease. Following the meeting, NIDDK staff met with investigators to develop future initiatives to support the development of therapeutics based on correcting protein misfolding.

On May 8–10, 2004, NIDDK, NHBLI, and the Office of Rare Diseases provided support for the meeting titled " Workshop on Bioiron in Thalassemia, Sickle Cell Disease, and Hemochromatosis," sponsored by the Center for BioIron at CHORI, Oakland, CA, through 1R13 DK065630-01. The workshop was attended by more than 30 registrants, with additional students and postdoctoral fellows from various California Bay Area universities and medical schools. The principal organizer of the workshop was Elizabeth C. Theil, Ph.D., an internationally recognized expert on the mechanisms of iron transport and iron overload. The purpose of the workshop was to enhance translational research in bioiron, including iron overload with hypertransfusion and malabsorption as the iron source, and to provide opportunities for collaborative research interactions for junior scientists in groups already engaged in basic/clinical research. The location was selected to take advantage of established foci of unusual strength in clinical and basic research and training in bioiron related to hemochromatosis or sickle cell disease and thalassemia and to begin to rectify underdeveloped potential to share common information on iron. Topics ranged from the interactions of liver iron and hepatitis C and mechanisms of iron chelation in iron overload to organ dysfunction in iron-overloaded patients with thalassemia. Participants engaged in a summary session where topics of future research interest were discussed, based on unresolved issues identified in the workshop. Staff members of the NIDDK and NHLBI were present during this discussion. Following the workshop, these staff members met to identify priority areas that might be addressed in future initiatives.

On June 20–22, 2004, NIDDK, NICHD, and ORD provided support for the Ninth International Workshop on Multiple Endocrine Neoplasias. The total registration at the meeting was 196 and there were 65 posters. Three keynote talks covered broad issues. Symposium 1 covered molecular biology of pituitary tumors with an emphasis on mechanisms of tumorigenesis. Symposium 2 covered thyroid tumors with a focus on long-term follow-ups of surgery indicating prevention and cure of medullary thyroid cancer in men2. Working groups met to discuss patient issues, tyrosine kinase inhibitor drugs, and organization of a pheochromocytoma consortium. The workshop abstracts have been published in the *Journal of Internal Medicine* 255:696–730, 2004.

In 2003, the NIDDK and NIBIB funded four grants in response to RFA DK-03-007, Noninvasive Measurements of Iron by Magnetic Resonance Imaging. A meeting of these grantees was held in September 2004, and considerable progress has been made in developing each of these projects. The diagnosis and management of patients with iron overload, including those with Cooley's anemia, would be improved greatly if physicians had ready access to a noninvasive, safe, and accurate way to measure body iron levels. It was apparent from the reports at the meeting that MRI technology has potential as a technology to measure body iron. Reasonably high quality images of stored iron have been obtained and, even at this stage, have begun in some cases to be useful clinically. A second meeting is planned for 2005.

**Activities with Rare Diseases Patient Advocacy Groups**

The Cooley's Anemia Foundation, NIDDK, NHLBI, CDC, and HRSA participated in the first inter-agency meeting focused on thalassemia held on September 17, 2004. The purpose was to learn about the activities in the different agencies to enhance integration and communication. One of the recommendations from this first interagency meeting is to establish a Thalassemia Working Group within the government for greater communication and cooperation.

On April 22, 2004, NIDDK and the Oxalosis and Hyperoxaluria Foundation held a Calcium Oxalate Stone Disease Taskforce Meeting in Bethesda, MD. The purpose was to formulate a research agenda for hereditary calcium oxalate stone disease, emphasizing translational research that will bring promising discoveries from the laboratory to a clinical research setting. Numerous recommendations emerged from this meeting for enhancement of research on improved diagnostics, epidemiology, pharmacologic treatment, microbial therapies, and gene therapy as well as strengthening of training for investigators in this area.

# NATIONAL INSTITUTE ON DRUG ABUSE (NIDA)

## Overview of Rare Diseases Research Activities

### Background

The National Institute on Drug Abuse (NIDA) provides national leadership and conducts and supports biomedical and behavioral research, health services research, research training, and health information dissemination addressing the prevention of drug abuse and treatment of drug addiction. NIDA plans, conducts, fosters, and supports a comprehensive program of research and research training relating to the causes, prevention, treatment, patterns, and consequences of drug abuse and addiction. Research is performed in NIDA's own laboratories and through contracts and grants made to scientific institutions and individuals. Training relevant to fundamental sciences and clinical disciplines of drug addiction is performed via institutional and individual research training awards and collaborations with other research institutes and Federal health agencies. NIDA conducts and fosters health information dissemination activities, including the collection and dissemination of research findings and related educational materials for health professionals, educators, and the public. In addition, NIDA coordinates with institutions, professional associations, and agencies both domestic and abroad that specialize in the treatment and research of drug addiction, specifically with the Substance Abuse and Mental Health Services Administration on services research issues as well as on other programmatic issues.

### Size of Drug-addicted Population

The abuse of opiates such as heroin and other narcotics exceeds 1 million persons and stimulants such as cocaine and “crack cocaine” exceeds 2 million; they are endemic in the United States (U.S. Office of National Drug Control Policy). However, even the lowest estimates from any source put addiction levels of these substances at figures well above the 200,000 threshold generally used for defining orphan products. Incidence and prevalence figures for addiction on controlled substances are difficult to estimate, as they vary from type of drug, community, and supply availability (generally a function of supply interdiction/law enforcement). There may be various drug addiction indications that affect less than 200,000 persons in the United States. The total cost to society of drug abuse and addiction in the United States has been estimated to exceed $484 billion per year.

### Drug Addiction as an Orphan Disease

Although drug addiction is a serious public health concern, it is a historical fact that drug addiction is treated as an orphan disease because the pharmaceutical industry rarely profits from marketing drugs for the treatment of drug addiction and there exists little or no incentive for pharmaceutical companies to pursue research and development of new treatment medications for this population. Although total numbers of persons afflicted may seem sufficient in the aggregate, unlike other disease states, many of these persons are not seeking treatment upon diagnosis. Therefore, the actual patient population is less than the total number of persons afflicted.

Additionally, many of these persons will be treated in publicly funded clinics where companies perceive reimbursement as modest or inadequate and perhaps subject to artificial cost controls. Finally, much of the U.S. treatment system is based on nonpharmacological treatment modalities.

A further complication is that some treatment agents may themselves be abusable and will be strictly controlled (witness methadone, classified as a Schedule II controlled substance for use in opiate maintenance therapy—some 900 U.S. clinics are licensed to dispense methadone and serve approximately 190,000 persons per year with a pharmaceutical market value of approximately $40 million per year). This is simply not an attractive market to most manufacturers based on projected return on investment when compared to nearly any other indication. Each of these points is well documented in the Institute of Medicine *Report on the Development of Medications for the Treatment of Opiate and Cocaine Addiction*, 1995, and all are well known to the pharmaceutical and market research industries. Therefore, while de jure opiate and cocaine addiction do not fit the definition of orphan products, de facto they certainly are treated as such. As an instructive example, consider the development and approval of levomethadyl acetate hydrochloride (trade name ORLAAM), an alternative to methadone for the treatment of opiate addiction. Despite the facts that human data on 6,000 subjects from government-sponsored studies were available for levomethadyl acetate hydrochloride and the government had a large supply of the compound available for anyone interested in obtaining a New Drug Application (NDA), no private sector entity attempted to finish the development of this compound until NIDA paid a contractor to do so. Similarly, the development of naltrexone was largely a NIDA-funded effort. Therefore, these products should be viewed as entirely "orphan-like" insofar as their ability to attract private sector sponsors.

**History of NIDA Rare Diseases Research**

Currently, there are four medications for the treatment of opiate addiction that have received orphan product designation. Each of these products was developed with substantial involvement by NIDA. These drugs are ORLAAM, naltrexone, buprenorphine, and buprenorphine combined with naloxone. ORLAAM, received NDA approval in 1993. Naltrexone, an opiate antagonist for use in detoxified patients, was approved in 1985. Currently, orphan exclusivity for ORLAAM and naltrexone has expired. Additionally, ORLAAM's distributor notified physicians that distribution of the product would be discontinued in 2004, due to poor sales in the United States and its withdrawal from European markets. ORLAAM's orphan product designation expired in 2000 and thus there is no legal requirement for a manufacturer to maintain the product in the U.S. market.

The opiate partial agonist buprenorphine and a combination of buprenorphine plus naloxone have also received orphan designation (see details below) and were approved for marketing in the United States on October 8, 2003. These products represent a major success as the FDA designated them both as orphan products. Buprenorphine became the first product to receive an orphan designation based on an economic rather than a population-based rationale, i.e., the product would not recoup their developmental expenses in 7 years of exclusivity in the U.S. market.

**Recent Scientific Advances in Rare Diseases Research**

The discovery of opiate receptors by NIDA-funded scientists in the 1970s opened a new era of neurobiological research that is ongoing today. Scientists continue to map brain receptor system types and subtypes, continuously gaining understanding of their structure and function. This information will allow the design of interventions (behavioral, chemical, and genetic) that may be useful in the treatment of a huge number of disorders of mankind, all of which are mediated in the brain.

A generation of research has shown that drug addiction is a complex biomedical and behavioral disease that affects those parts of the brain that underlie and mediate human emotions. Evolution in scientific research has demonstrated that both depression, an illness commonly observed in drug-addicted individuals, and drug addiction are brain diseases that can and often should be treated with medicine.

The role of a medication is to reestablish normality to brain function and behavior so that the addicted patient has the *opportunity* for rehabilitation through counseling, psychotherapy, vocational training, and other therapeutic services. While the mechanisms of many central nervous system disorders are still to be elucidated, scientists working in the field of drug abuse have now identified and cloned the putative site of action in the brain for every major drug of abuse. In addition, recent application of microarray technology to characterize drug effects on gene expression has identified intracellular proteins that are altered by drugs of abuse after their initial receptor interactions. Thus, the potential to develop new treatments is enormous.

**Rare Diseases Research Initiatives**

As described in the history section above, NIDA considers medications for the treatment of addiction on controlled substances to be de facto orphans. Thus, the development of medications for the treatment of addiction could be considered rare diseases research within the context of an urgent public health need with a wholly inadequate private sector response. Therefore, NIDA's Medications Development Program effort may (until facts prove otherwise) be considered as part of a rare diseases research initiative.

**The Functions of the Division of Pharmacotherapies and Medical Consequences of Drug Abuse**

In 1990, the Medications Development Division (MDD) was established in NIDA. In 1999, the MDD become the Division of Treatment Research and Development (DTR&D). In 2004, DTR&D became part of the Division of Pharmacotherapies and Medical Consequences of Drug Abuse (DPMC). The functions of MDD within the new division remained the same; namely, DPMC conducts studies necessary to identify, develop, and obtain FDA marketing approval for new medications for the treatment of drug addiction and other brain and behavior disorders; develops and administers a national program of basic and clinical pharmacological research designed to develop innovative biological and pharmacological treatment approaches; supports training in fundamental sciences and clinical disciplines related to the pharmacotherapeutic treatment of drug addiction; collaborates with (a) the pharmaceutical and chemical industry in the

United States and other nations, and (b) the Federal medications development programs of other institutes and entities; and works closely with the FDA in assuring that research designed to show the clinical efficacy of new compounds is evaluated and approved in the most expeditious manner possible.

The DPMC operates within the larger context of a NIDA-wide Medications Development Program that incorporates basic research discoveries from both the intramural and extramural communities in the quest to develop new pharmacological treatments. Application of research results from the intramural and extramural community allows DPMC to have access to the latest theoretical bases and an opportunity to test new hypotheses in controlled clinical settings. As physicians now have a choice of several different FDA-approved products for treating opiate addiction (methadone, buprenorphine, buprenorphine/naloxone, and naltrexone) and no FDA-approved products for treating addiction to stimulants (e.g., cocaine or methamphetamine), NIDA's efforts are currently shifting toward a greater emphasis on discovery and development of medications for treating stimulant addiction (cocaine, methamphetamine, nicotine) and, as of 2003, cannabis addiction. Clinical trials in this area have focused on medications that are already marketed for other indications, and substantial efforts are also being devoted to the discovery and development of novel compounds that may specifically address the problem of stimulant addiction through attempts to collaborate with the pharmaceutical industry. Efforts are also directed toward supporting, through grants and contracts, synthesis of novel compounds for screening and pharmacological testing.

Significant areas of research and development are summarized below:

**1. Opiate Addiction Treatment**

**Buprenorphine/Buprenorphine-Naloxone Combination:** A major milestone and achievement for NIDA's Medications Development Program and for Reckitt Benckiser Pharmaceuticals, Inc., (NIDA's collaborator in a Cooperative Research and Development Agreement) was the October 8, 2002, FDA approval for the marketing of two new products for the treatment of opiate addiction. These two new products, known under the trade names Subutex and Suboxone, represent new tools in the arsenal of anti-addiction medications. Both have been designated as orphans, based on the expectation that these products will not recoup their developmental expense during their period of U.S. marketing exclusivity. Marketing of these products began in January 2003 but is subject to certain restrictions imposed by U.S. law and regulations. Nevertheless, these products may, under the conditions specified in law and regulations, be prescribed in a variety of settings, including physician's offices.

Subutex and Suboxone now join methadone as medications that will be available for the treatment of heroin and other opiate addiction. They offer a broader array of options to physicians and patients and should expand treatment availability.

These products represent the culmination of several years of research and development between NIDA and Reckitt Benckiser Pharmaceuticals, Incorporated. The unique pharmacology of Subutex (buprenorphine) and Suboxone (buprenorphine combined with naloxone) and the statutory changes enacted by the Drug Addiction Treatment Act of 2000, as contained in P.L. 107-273, "The Children's Health Act of 2000," permits these products to be prescribed by appropriately trained physicians in settings other than the existing, but limited, Opiate Treatment Programs (OTPs). It is hoped that this will translate into an increase in treatment availability across the United States. A wider dispersal of new treatment settings should follow the introduction of these products to the market. Additionally, patients who either have no OTP programs available or who cannot avail themselves of these programs will have another option for treatment. As of the date of this report, the Substance Abuse and Mental Health Services Administration (SAMHSA), U.S. Department of Health and Human Services, reports that 6,202 physicians have taken the training required by law to prescribe these two medications and 3,905 have registered as potential providers.

**Depot Naltrexone:** Naltrexone, a marketed long-acting, orally effective opioid antagonist, was approved in 1983 for the indication of blocking the pharmacological effects of exogenously administered opiates. It is an adjunct to the maintenance of the opioid-free state in detoxified, formerly opioid-addicted individuals.

One of the major obstacles to the success of naltrexone has been patient compliance with therapy. Naltrexone must be taken at least three times per week and has no effect other than to block the effects of heroin, a drug that the patient is not supposed to use. Because of this, many patients forget to take or stop taking their medication. Therefore, the greatest success with naltrexone has been in the limited population of highly motivated formerly opiate-addicted patients.

During 1999, NIDA completed, via a Small Business Innovative Research (SBIR) grant to Biotek, Inc., the production and preclinical testing of a batch of 120 doses of depot naltrexone. These doses are designed to last 30 days when administered subcutaneously in humans and to produce a blood level of about 2–3 ng/ml (which will be relatively constant over this period). This product has been shown, in an inpatient clinical study, to block subjective responses to heroin challenges at 12–25 mg. This study was completed in 2000 and showed that it was possible to block 25 mg heroin challenges up to 5 weeks after depot injection. A two-site outpatient double-blind study was designed to test the product in a real-world setting. This outpatient study began in November 2000 and was completed in 2003. The results were reported at a scientific meeting in June 2004 and are pending publication. The results indicate a significant effect of the depot preparation to limit relapse to heroin in the trial.

Additionally, another investigational formulation of a sustained release formulation of naltrexone supplied by Alkermes, Inc., has undergone clinical trials at NIDA's Intramural Research Program. This study provided information on the safety and duration of effect of this potential treatment product. The oral form of naltrexone was approved in 1994 for the treatment of alcohol abuse; the depot preparation may also be of value for the treatment of that disease.

Alkermes has reported that their dosage form of depot naltrexone reduces heavy drinking behavior in males (but not females) and that the company intends to pursue further development of their dosage form. They plan a submission to FDA for approval within the next 2 years.

Thus, the feasibility of a sustained release formulation of naltrexone for the treatment of opiate and alcohol addiction is moving rapidly from concept and clinical testing toward potential regulatory approval and marketing.

**2. Cocaine Addiction Treatment**

Several small studies of potential cocaine addiction treatment agents have been completed and are in various stages of data analysis. Clinically significant findings will be followed up in larger controlled trials as warranted. In addition, NIDA continues to work toward the discovery and development of new molecular entities, such as kappa opioid antagonists, for cocaine addiction treatment.

**Ondansetron:** A 5HT3 antagonist that can block dopamine release and increase GABA tone has been shown to be efficacious in reducing cocaine use at dose of 8 mg/day in a recently completed NIDA study. The consensus from the data analysis and safety data review was to proceed forward with a follow-up phase IIb study to explore and confirm the results of the phase IIa study with two doses of ondansetron—8 mg and 16 mg—and placebo. This study will be conducted in roughly 200 patients.

**Quetiapine:** An atypical neuroleptic has been shown to decrease cocaine use and craving in co-morbid bipolar patients. A phase I safety trial is under way. A phase II proof-of-concept study with two doses of Quetiapine and placebo is planned.

**Cabergoline:** A long acting dopamine agonist that showed a trend for efficacy in reducing cocaine use in a small pilot study is being studied for confirmation in a large phase II study. This study is completed and data are being analyzed.

**Modafinil**: A nondopaminergic stimulant has been shown to reduce cocaine use in a pilot study (Dackis 2004). It is currently being studied in a multisite trial for confirmation of efficacy. Recruitment for this study is about to begin.

**RPR:** A CCK-B antagonist that modulates dopamine release and is currently in phase I safety trials.

**Tiagabine**: A GABA uptake inhibitor has been shown to decrease cocaine use in two pilot studies. A multisite trial has been recently completed. Data analysis is under way.

**Atomoxetine**: A norepinephreine uptake inhibitor approved for ADHD is currently in phase I trials for safety in cocaine-abusing subjects. Pending safety data, a phase II study will be planned for study in co-morbid cocaine subjects with ADHD.

**Baclofen**: A GABA-B agonist has been shown in a pilot trial to reduce cocaine use in heavy users. It is being studied in a multisite trial to confirm earlier findings.

**Disulfiram:** There is a growing body of evidence generated by NIDA grantees concerning the potential use of disulfiram in the treatment of cocaine addiction. Disulfiram (Antabuse), marketed as aversive therapy for treating alcoholism, is also showing promise in the treatment of cocaine addiction. Several NIDA-sponsored studies conducted at Yale University documented interaction of disulfiram with cocaine in humans. Pharmacokinetics studies showed that disulfiram increases plasma concentrations of cocaine and potentates physiological-cardiovascular responses to cocaine. Three efficacy trials conducted with different populations of cocaine-addicted individuals suggest that disulfiram in combination with each of three different therapeutic interventions (cognitive behavioral treatment, 12-step facilitation, or clinical management) might be effective in treating cocaine addiction. In cocaine-alcohol abusers disulfiram treatment showed sustained effect on reduced cocaine and alcohol use 1 year after cessation of the therapy. Disulfiram treatment of cocaine-abusing opioid-addicted patients maintained on methadone resulted in significant decrease of the amount and frequency of cocaine use. A preliminary study showed that disulfiram also decreases cocaine use in cocaine-opioid addicts maintained on buprenorphine.

NIDA is currently sponsoring three large outpatient clinical trials with disulfiram as the treatment for cocaine addiction: (1) study on 160 opioid-cocaine-addicted patients maintained on methadone, conducted at Yale University; (2) study on 180 opioid-cocaine-addicted patients maintained on buprenorphine, conducted at Yale University; (3) study evaluating disulfiram and naltrexone alone and in combination in the treatment of 208 alcohol-cocaine-addicted individuals, conducted at the University of Pennsylvania. All these studies include some form of behavioral or cognitive therapy and drug counseling. They are monitoring not only use of cocaine but also opiates or alcohol. Finally, NIDA is planning a clinical pharmacology/safety study of the interactions between disulfiram and iv-administered cocaine, prior to launching large-scale phase III multi-center trial with this medication.

**GBR 12909** (**Vanoxerine**): Major neurochemical effects of cocaine include release of dopamine (DA), serotonin, and noradrenaline via a transporter-mediated exchange mechanism. There is considerable evidence that the initiation and continuation of cocaine use is associated with the effects of the drug on the dopaminergic, serotonergic, and noradrenergic modulation of the central nervous system (CNS) function. Animal studies suggest that the mesocorticolimbic dopaminergic pathways are important mediators of cocaine's reinforcing and addictive properties. Cocaine binds to these transporters and blocks the removal of these neurotransmitters from the synaptic gap. The neurobiological mechanisms underlying the effects of cocaine are not well understood. Preclinical studies indicate that cocaine's blockade of the DA transporter plays a key role in producing cocaine's addictive and reinforcing effects. Primate and nonprimate studies have shown that GBR 12909 has a strong affinity for the DA transporter. GBR 12909 is a high-affinity, selective, and long-acting inhibitor of DA uptake that produces a persistent and noncompetitive blockade of DA transporters and substantially reduces cocaine-induced increases in extracellular mesolimbic DA. In addition, GBR 12909 has a higher affinity than cocaine for the DA transporter. Ongoing research is searching for a dopamine-sparing cocaine antagonist that might be developed as a pharmacological treatment to block cocaine from acting at the transporter level to produce its reinforcing effects. GBR has been postulated to act by binding only to precise sites on the

dopamine transporter that are required for cocaine binding and making available the sites where DA binds to the transporter.

A phase I clinical study was conducted in support of an Investigational New Drug (IND) application filed by NIDA. The main objectives of this study were to determine the safety, tolerance, and pharmacokinetics of multiple escalating dosages of oral GBR 12909 in healthy volunteers. In addition, PET scans measuring the occupancy of the DA transporter by GBR 12909 were obtained. The occupancy scan results are being correlated with the safety data to determine an optimal oral dose of GBR 12909.

The study report from the Phase I (healthy volunteer) study showed 30–40-percent dopamine transporter occupancy at the 100 mg dose level. Based on primate data showing equivalent levels of occupancy at doses reducing cocaine self-administration, this may be clinically meaningful in cocaine treatment. Consultants reviewed the study in October 2001. The consultants recommended a follow-up study in cocaine-addicted patients to address the safety and other metabolic issues that were raised in the first study in planning for a cocaine interaction study. Phase I studies in cocaine-addicted subjects have begun.

**Dopamine Agonists:** The activation of the dopaminergic reward system in the brain appears to be the principal neurochemical mechanism involved in the addiction to stimulants such as cocaine and amphetamine. Chronic abuse of these drugs results in dopamine deficiency in the brain, which has been hypothesized to lead to craving for stimulants, depression, anhedonia, and dysphoria.

Most recently, studies in rodents, and to a lesser extent in monkeys, have differentiated the roles of D1 and D3 receptors with regard to cocaine. The D1 system may inhibit the effects of cocaine, while the D3 system may block conditioned cues. Compounds that affect both systems are under study.

**Kappa Opioid Antagonists:** While the discovery of a selective kappa opioid antagonist for potential use in preventing relapse to opiates has been a goal of NIDA for several years, the recent discovery and evaluation of JDTic—a highly selective kappa antagonist synthesized by a NIDA chemist—has provided a rationale for effectiveness in preventing relapse to cocaine. Interestingly, JDTic was shown to prevent stress-induced reinstatement of responding to cocaine in a rat model of cocaine relapse. The compound is currently undergoing initial preclinical safety testing to determine its developability as a medication.

**Glucocorticoid and Corticotropin Releasing Factor (CRF) Antagonists:** Studies have shown that cocaine causes the release of stress hormones known as glucocorticoids in both rats and humans. There is some evidence from rat studies that glucocorticoid antagonists and CRF antagonists reduce cocaine self-administration in a dose-related manner. NIDA will follow up on these basic research findings with additional studies aimed at developing a potential treatment for cocaine addiction. DPMC is attempting to obtain CRF antagonist compounds from pharmaceutical company sources.

**Immunology:** During 1998, NIDA sponsored a meeting on the potential of utilizing peripheral blockers for prevention and treatment of cocaine addiction. The ability to block cocaine's entry

into the brain or decrease its rate of entry (and thus attenuate the "high" produced) was discussed. Several approaches (active and passive immunization, catalytic antibodies) were actively explored. One of these theoretical constructs has now been translated into actual therapeutic entities that are currently at various stages of research and development as listed below.

Researchers funded by NIDA's DPMC reported that they have successfully immunized rats against many of the stimulant effects of cocaine. Cocaine was prevented from entering the brain when rats were "vaccinated" with a substance that triggers the body to produce antibodies to cocaine. These antibodies then acted as biological "sponges" to which cocaine binds, thereby reducing the amount available in the blood to reach the brain. The results of this research are presented in "Suppression of Psychoactive Effects of Cocaine by Active Immunization" in the December 14, 1995, issue of *Nature*.

Researchers Kim Janda, Ph.D.; Rocio Carrera, M.A.; George Koob, Ph.D.; and colleagues at The Scripps Research Institute demonstrated a greater than 70-percent reduction in cocaine uptake in the brains of rats inoculated with the antibody-producing compound as compared to a group that was not inoculated. Researchers designed the compound so that the antibodies produced would respond specifically to the cocaine molecule yet not affect normal brain chemistry.

In the study, Dr. Janda and colleagues used an "active immunization" approach by developing a substance that when administered to rats would trigger the immune system to produce antibodies that are specific for the cocaine molecule. The researchers inoculated the rats over a 35-day period and then tested their responses to cocaine. The immunized animals showed significantly lower responses to the stimulant effects of cocaine than control animals because the immunization prevented much of the cocaine from getting to the brain. Cocaine concentrations in the brain tissue of the immunized animals were found to be dramatically less than the concentrations of cocaine in brain tissue of controls.

Other immunotherapy research for drug abuse treatment has explored the use of catalytic antibodies and other external agents that can be used to treat cocaine addiction. The research reported in *Nature* differs by inducing the production of antibodies that remain in the bloodstream for an extended period of time and block cocaine's effects after it is used.

Another vaccine, currently owned by Xenova, a United Kingdom company, links a carrier protein to cocaine, resulting in an antigen that induces antibody formulation. This vaccine generates antibodies that can retain cocaine in the bloodstream and allow naturally occurring cholinesterases to convert cocaine into inactive metabolites. NIDA has supported the initial research via a SPIRCAP grant. Thirty-four subjects, all former cocaine abusers, completed the initial phase I study in the United States, and the vaccine was found to be clinically safe and to produce substantial levels of anti-cocaine antibodies that persist for at least 3 months following the final vaccination. An additional study examining the extent to which the antibody can block the effects of administered cocaine was also funded under the SPIRCAP award.

A phase II safety and immunogenicity study funded through a NIDA grant to Dr. Thomas Kosten at Yale University started in 2002. The company plans to continue development of the vaccine.

Dr. Michael Owens, at the University of Arkansas for Medical Sciences in Little Rock, presently receives NIDA funding to develop a new generation of monoclonal antibody-based medications for treating drug abuse ("Immunotherapy for Drug Abuse," R01 DA07610, "Antibody-Based Therapy for Methamphetamine Abuse," R01 DA11560, and "Preclinical Testing of Antibody Therapy for Methamphetamine Abuse", P01DA14361). This research is focused on treatments for methamphetamine, ecstasy (MDMA), and phencyclidine (PCP) abuse. Additionally, NIDA awarded an STTR grant in FY04 to Inflexion Therapeutics for the advanced development of the anti-PCP monoclonal antibody invented by Dr. Owens. This project focuses on the production of anti-PCP monoclonal antibodies in tobacco plants and clinical development of the antibody. These medications function as pharmacokinetic antagonists and are designed to reverse the effects of drug overdose and/or help blunt the reinforcing effects of drugs of abuse. Because of the unique pharmacological profile of these new medications, they would be well suited for use with other more conventional chemically based medications and treatments, such as behavioral modification, to aid in the long-term recovery from drug addiction.

**Cocaine "Receptor" Imaging Studies:** In addition to the categories of compounds being tested as described above, a new and potentially useful technology is being investigated as to its value for predicting efficacy of potential cocaine treatment medications. Research in the field of structure-activity relationships has revealed highly selective and potent binding ligands for the dopamine transporter. NIDA intramural researchers have identified three "generations" of such compounds, with each succeeding generation being more selective and potent than the previous one. RTI-55, the first potent compound, was shown to be an effective *in vivo* labeling agent in animal studies and was subsequently examined in human imaging studies by SPECT. A second compound, RTI-121, was found to be more selective for the dopamine transporter but had a higher apparent lipid solubility and exhibited lower specific to nonspecific binding *in vivo*. NIDA researchers are testing new compounds and are also utilizing some older compounds (e.g., WIN-35,428) in brain imaging studies. Procedures have been developed for estimating the occupancy of transporter sites *in vivo*. Dopamine transporter imaging studies of cocaine abusers have been completed (see section on GBR 12909). This technology may make it possible to estimate the effectiveness of a potential treatment compound or regimen by correlating receptor occupancy (as shown in imaging studies) with actual clinical results. NIDA will continue to follow this line of research.

Additionally, NIDA is participating in an effort with NIMH, NINDS, and NIAAA to develop appropriate imaging ligands that will be essential to the study of many brain and CNS conditions as well as the effects of various treatments.

### 3. Methamphetamine Addiction Treatment

Methamphetamine is a potent psychomotor stimulant that has gone through episodic periods of widespread use and abuse in the United States. Cocaine abuse and addiction surpassed use of methamphetamine in the 1970s and 1980s, but methamphetamine abuse and addiction have been reappearing in some regions of the United States and are widespread in Western U.S. cities such as San Francisco, Denver, Phoenix, and Los Angeles. According to the 2003 National Survey on Drug Use and Health, approximately 12.3 million Americans ages 12 and older reported trying methamphetamine at least once during their lifetimes, representing 5.2 percent of the population

ages 12 and older. Approximately 1.3 million (0.6 percent) reported past year methamphetamine use and 607,000 (0.3 percent) reported past month methamphetamine use.

There are no accepted treatment medications for methamphetamine addiction or abuse. As a result, NIDA has developed a Medication Discovery Program for methamphetamine and is funding a number of extramural and intramural studies to develop medications to treat methamphetamine abuse.

**Ondansetron:** There is evidence that selective serotonin 5-$HT_3$ receptor antagonists attenuate behavioral responses to d-amphetamine and methamphetamine, suggesting that 5-$HT_3$ receptors modulate brain dopamine in animals. This action of 5-$HT_3$ receptor antagonists may reduce the rewarding effects of abused substances. Ondansetron is a selective 5-$HT_3$-receptor antagonist. Ondansetron decreases stimulated dopamine release and has been shown to reduce the development of behavioral tolerance and sensitization to cocaine following a period of acute and chronic withdrawal. It has also been suggested that 5-$HT_3$ antagonists may reduce discomfort or post-cessation anxiety following psychostimulant withdrawal. These data prompted us to test whether Ondansetron might reduce cocaine-mediated reward and ameliorate post-cessation anxiety symptoms following cocaine use cessation. NIDA and UCLA established a Methamphetamine Clinical Trials Group (MCTG). The MCTG conducted a phase II double-blind, placebo-controlled dose-response trial with Ondansetron. The study was completed in 2003. There were no significant effects for any Ondansetron dose compared to placebo.

**Selegiline:** A selective monoamine oxidase-B (MAO-B) inhibitor that is used to treat patients afflicted with Parkinson's disease. Parkinson's disease is a neurological disorder that results from brain dopamine depletion. Selegiline improves brain dopamine levels and helps restore and maintain functionality in patients with Parkinson's disease. Methamphetamine produces its behavioral and cognitive effects by affecting dopaminergic mechanisms in the midbrain. Specifically, methamphetamine causes dopamine release and blocks its reuptake in dopaminergic nerve endings. Excess dopamine in brain synapses stimulates the midbrain reward centers but eventually dopamine is depleted. NIDA decided to test selegiline for its potential to reduce methamphetamine craving by restoring the depleted dopamine. A phase I interaction trial to determine the safety of selegiline in the presence of methamphetamine was completed in November 2003. No safety concerns were identified. A phase II, double-blind, placebo-controlled clinical trial of selegiline for methamphetamine relapse prevention is scheduled to start January 2004.

**Aripiprazole:** An atypical neuroleptic drug that has been approved by the FDA to treat schizophrenia and bipolar mania. It is a functional *antagonist* at dopamine $D_2$ receptors in a *hyper*dopaminergic environment, a functional *agonist* at dopamine $D_2$ receptors in a *hypo*dopaminergic environment, and a serotonin *antagonist* at serotonin 5-$HT_{2A}$ receptors and *partial agonist* at serotonin 5-$HT_{1A}$ receptors. It has moderate affinity for $alpha_1$-adrenergic and histamine ($H_1$) receptors. Long-term methamphetamine abuse results in schizophrenia-like symptoms. By extension aripiprazole may have potential as a methamphetamine abuse therapeutic. NIDA is currently conducting a Phase I safety interaction study with aripiprazole and methamphetamine at UCLA and NYU.

**Lobeline:** A derivative from Indian tobacco plants. It stimulates a subclass of nicotine receptors and thus was tested in the clinic for its potential as a smoking cessation therapeutic. Additionally lobeline redistributes dopamine in nerve terminals by preventing dopamine uptake into synaptic vesicles without inhibiting MAO-B. In contrast, methamphetamine enters synaptic vesicles and inhibits MAO-B. Studies in rats revealed that lobeline decreases methamphetamine self-administration without affecting the rats' ability to self-administer sugar water. These data suggest that lobeline may reduce acute rewarding effects of methamphetamine and corresponding abuse liability. NIDA is collaborating with a pharmaceutical company to conduct a phase I double-blind, placebo-controlled, ascending-dose pharmacokinetic, safety, and tolerability study of lobeline with an eye toward testing it as a methamphetamine abuse therapeutic.

**Reserpine:** An alkaloid extracted from the roots of the plant *Rauwolfia serpentina*. It's a monoamine depleter that works at the synaptic vesicle, the same site of action as lobeline. Reserpine has been used since the 1950s to treat mild to moderate hypertension. In addition, it was one of the first antipsychotic drugs. Its present use in psychiatry is almost obsolete, as other neuroleptic drugs are more effective. NIDA is completing a phase I single-dose study with reserpine at UCSF. The data are currently being analyzed. An earlier proof-of-concept study with reserpine for cocaine abuse did not show efficacy. Further studies of reserpine for methamphetamine abuse are a low priority at this time in view of this finding.

**Bupropion:** A medication that has been approved to treat depression and promote smoking cessation. It is a dopamine uptake inhibitor that is well tolerated and has a good safety record. NIDA is testing bupropion for its ability to alleviate the dysphoria seen in early abstinence and reduce methamphetamine craving and relapse to drug using. Currently we are conducting a phase II, double-blind, placebo-controlled study of Bupropion.

**Modafinil:** A nondopaminergic stimulant used to treat narcolepsy. It is hypothesized that this effect is accomplished by modulating the glutamate/GABA system. Recently it has been shown that modafinil blunts cocaine euphoria under controlled conditions and improves clinical outcome in cocaine-addicted patients receiving standardized psychosocial treatment. Methamphetamine, like cocaine, is a stimulant. Thus NIDA is endeavoring to evaluate the potential of modafinil as a methamphetamine abuse therapeutic. NIDA is planning a phase I safety trial, as requested by FDA, prior to conducting a phase II trial.

**Topiramate:** A fructopyranose derivative that is approved for treating seizure disorders. In a clinical trial it was found to be superior to placebo at improving drinking outcomes in alcohol-addicted individuals. Data from a pilot proof-of-concept study suggest that topiramate may be a useful medication for preventing relapse to cocaine use. As both cocaine and methamphetamine are stimulants it is not unreasonable to hypothesize that topiramate may be a useful medication for preventing relapse to methamphetamine use. To that end NIDA is providing grant support to conduct a phase I safety interaction study between topiramate and methamphetamine.

**GBR 12909:** A potent dopamine uptake inhibitor that has been shown in non-human primate positron emission tomography (PET) imaging studies to block amphetamine-induced dopamine release. Currently, GBR 12909 is being tested in phase I interaction studies for cocaine abuse. NIDA is planning a phase I safety interaction study with methamphetamine. Barring any safety

concerns NIDA would test GBR 12909 in an outpatient proof-of-concept study for methamphetamine relapse to using prevention.

**4. Medications Development for Cannabis-related Disorders**

The treatment of cannabis-related disorders (CRDs) is an issue of great public health concern. Currently, marijuana is the most commonly used illicit drug in the United States with recent estimates from the SAMHSA of 14.6 million users in the past month, with particularly heavy use occurring in adolescent populations (over 20 percent of all high school seniors). Approximately 2.4 million people use marijuana for the first time every year and two-thirds of them are between 12 and 17 years of age. In addition, of the 3.5 million people who met criteria for past-year cannabis abuse or addiction in 2001, more than two-thirds were between the ages of 12 and 25 years. An estimated 852,000 individuals reported marijuana as the specific substance for which they received their last or current treatment among persons who received treatments in the past year, and approximately one-half of those individuals were 25 years old or younger.

Sufficient research has been carried out to confirm that the use of cannabis can produce serious physical and psychological consequences. The consequences of cannabis use may be due to the acute effects of the drug or due to the chronic exposure that may ultimately produce addiction. The use of a large amount in a short period of time may induce hallucinations, delirium, and other perceptual manifestations similar to a psychotic episode. Chronic users of cannabis may experience difficulty in stopping or controlling drug use, develop tolerance to the subjective and cardiovascular effects, and eventually present withdrawal symptoms after sudden discontinuation of use.

Unfortunately, there is currently no effective pharmacological treatment for CRDs and there is very limited research focused on the identification and development of medications to treat these disorders. Drug abuse treatment research as a whole has rarely focused on the treatment of CRDs. One indicator is the fact that there are no published randomized controlled clinical trials to evaluate pharmacotherapies for CRD as the primary outcome.

There are multiple reasons why it is timely to develop medications to treat CRDs. First, there are newly marketed medications available whose mechanisms of action may have potential therapeutic effects on the clinical manifestations of CRD. Second, the recent discovery of an endogenous cannabinoid system with specific receptors and endogenous ligands, the availability of genetically engineered knockout mice that lack functional cannabinoid receptors to study genetic predispositions to the effects of cannabinoids, and the subsequent development of reliable preclinical models to study the rewarding and addiction-producing effects of THC all provide understanding of the basic therapeutic mechanisms. Last, there is the discovery and development of new chemical entities, some of them already being investigated at the clinical level, that target the cannabinoid system and have the potential for therapeutic benefit. All these factors are setting the stage for the development of medications to treat CRDs.

## 5. New Research Projects for Medications Development for Cannabis-related Disorders

Based on the needs as described above, NIDA funded several new research grants through RFAs, the goal of which was the development of safe and effective medications for the treatment of CRDs. Preclinical and clinical studies are focusing on the treatment of marijuana, hashish, or other cannabis derivatives use disorders. Medications studied under this RFA are aiming to treat cannabis use disorders, such as abuse and addiction, or cannabis-induced disorders, such as intoxication, delirium, psychosis, and anxiety. They are also focusing on the specific symptoms of the disorder such as withdrawal, craving or relapse, complications such as cognitive impairment and sleep disorders/disruption of normal rhythms, or the clinical surrogates of their use such as depression and other mood disorders.

The rationale for choosing the medication(s) to be investigated have been based on a top-down approach, a bottom-up approach, or both approaches combined. The top-down approach is the testing of marketed medications that are available for other indications and may be promising candidates for the treatment of CRDs. For example, an FDA-approved antidepressant has been chosen as a target medication. The bottom-up approach involves the identification and testing of new chemical entities that because of their chemical characteristics and mechanism of action are candidates being developed specifically for CRDs.

## 6. Consequences of Drug Addiction

Currently, the program of research on medical consequences of drug abuses and co-occurring infections resides within the DPMC. As a result of the reorganization that created DPMC, critical research in the area of medical consequences of drug abuse will become a focus for NIDA. As described below, these studies involve marketed medications but typically will not be performed by the public sector. Under this program, the supported studies may be categorized into four major programs of research that are in various stages of development and progress. These are: (1) Metabolic and Endocrine Disorders of HIV/AIDS and Drug Abuse; (2) Pharmacokinetic/pharmacodynamic Drug-Drug Interactions Between Medications Used in the Treatment of Drug Addiction (e.g., methadone, newly FDA-approved buprenorphine), Infections (HIV, HCV, TB), and Mental Disorders; (3) Medical and Health Consequences of Chronic (long-term) Use/abuse of Licit and Illicit Drugs of Abuse and Co-occurring Infections (HIV, HCV, TB, STDs, and others); and (4) Oro-Maxillary Complications (such as facial and dental injuries) associated with Drug Abuse and Co-occurring Infections. New avenues of research are planned that fit within these major themes: (1) metabolic and endocrine disorders of HIV/AIDS among drug abusers, (2) directly observed therapy for HIV infected drug abusers, (3) hepatitis C, (4) issues in the medical management of HIV/HCV co-infections among drug abusers, (5) mini-symposium on TB among drug abusers, and (6) role of hormones and nutrition in drug abusers co-infected with HIV and HCV.

Injection drug use and sexual contact among users is a highly correlated vector in the spread of HIV, hepatitis, and tuberculosis. This creates a public health problem of enormous magnitude. The medical consequences may range from effects on the brain leading to drug addiction causing brain disease to effects on almost every physiological/organ system, including the central and peripheral nervous systems, cardiovascular, endocrine/hormonal, pulmonary/respiratory, renal,

hepatic/metabolic, reproductive, immune, and other systems. For example, stimulants such as cocaine and methamphetamine (met, speed, or ice) increase the heart rate while constricting the blood vessels; in susceptible individuals, these two actions together set the stage for cardiac arrhythmias and strokes. Cocaine use decreases the blood flow to the brain, increases the heart rate, and elevates the blood components that promote clotting—effects that can lead to stroke or heart attack even in those not otherwise at risk for these serious cardiovascular events. NIDA-funded research also shows that chronic cocaine use is associated with left ventricular dysfunction and increased calcium deposits in the coronaries of HIV-infected African Americans and that its use may also facilitate the entry of HIV into brain cells, leading to cognitive and memory impairment. The club drug methylene-dioxy-methamphetamine (MDMA), also known as ecstasy), which many users mistakenly believe to be safe, has caused malignant hyperthermia, permanent kidney damage, and death. MDMA also damages serotonin nerve fibers in the brain. Heroin can cause a life-threatening kidney condition called focal glomerulosclerosis. Opiate (heroin) use is associated with consequences ranging from nausea and constipation to renal, dental, and orofacial complications. PCP (phencyclidine, or angel dust) decreases heart rate and blood pressure, triggers violent aggression, and may trigger muscle contractions strong enough to break a bone. The use of the most-abused illicit drug in the world, marijuana, that is often perceived by many as innocuous, is also associated with consequences ranging from memory, cognitive, and motor problems in young as well as adult individuals to possible lung cancer in chronic marijuana smokers (see Clinical Consequences of Marijuana in a special supplement to *the Journal of Clinical Pharmacology*, Vol 42, no.11, Nov 2002).

Injecting drug use further promotes blood clots, severe skin infections, and blood-borne infections including life-threatening endocarditis, viral hepatitis, and HIV/AIDS. Abuse of some drugs is associated with impulsive sexual activity that elevates individuals' risks for acquiring and transmitting HIV/AIDS and other STDs.

In a relatively new area of research at NIDA, data show that nutrition may play an important role in HIV disease progression. Preliminary research shows that drug abusers with inadequate nutrition, particularly with suboptimal levels of antioxidant micronutrients such as selenium and zinc, are at high risk of mortality if they are also co-infected with HIV/AIDS. Clinical trials are under way to determine if supplementation with selenium, zinc, and other antioxidant micronutrients would slow the progression of HIV/AIDS disease. This research would have worldwide implications, such that underdeveloped countries where poor people cannot afford expensive antiretroviral therapy would benefit from an inexpensive treatment modality to slow disease progression and improve the quality of life.

Research also shows that hepatitis C virus (HCV) is another blood-borne pathogen that is easily transmitted through contaminated drug injection paraphernalia. Further, both viruses, HCV and HIV, frequently coexist because of common routes of transmission. Hepatic injury seems to occur in HIV/HCV co-infection through the induction of a novel signaling pathway that is cooperatively activated by specialized protein molecules, known as HCV E2 and HIV gp120, thereby providing a rationale for therapeutic interventions. NIDA continues to support a wide spectrum of research on epidemiology, natural history, underlying pathogenesis, prevention, and treatment of HIV/HCV co-infections among drug abusers.

## Drug-Drug Interactions

Research shows that some illicit drugs and drug abuse medications can interact with medications used for treating diseases, resulting in possible loss of efficacy and adverse effects. For example, an interaction can occur between methadone and the protease inhibiting drugs that are currently the most effective treatments for HIV infection. The result can be ineffectiveness and increased toxic side effects from one or both drugs. In some cases, the presence of a protease inhibitor has inhibited the metabolism (processing) of methadone sufficiently to cause patients to go into withdrawal. The identification of such interactions and development of alternative regimens is a high NIDA priority under a new program of Research on Drug-Drug Interactions**.**

Due to the lack of pharmaceutical industry interest in developing new medications to treat addiction to controlled substances, NIDA has been substantially involved in the development of nearly all such medications since the Institute's inception in 1974.

## NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES (NIEHS)

### Overview of Rare Diseases Research Activities

The National Institute of Environmental Health Sciences (NIEHS) supports basic research into the fundamental mechanisms of how environmental exposures interact with the human body to produce disease and dysfunction. This research on molecular pathways and environmental interaction has also yielded insights into the basic mechanisms involved in the pathogenesis of rare diseases and conditions. This work continues, with several related announcements either ongoing or nearing the award stage. Applications are currently solicited for studies of environmental influences in the development of amyotrophic lateral sclerosis (a focus of the Program Announcement, Gene/Environment Interactions in Neurodegenerative Disease). In addition, the new program of grants on metabolomics in environmental health science (using metabolomics technologies to study metabolic and regulatory pathways that are perturbed by environmental exposures) will be awarded soon and will provide insights on a wide range of cellular mechanisms by which environmental exposures influence disease.

### Parental Exposure to Specific Chemicals May Lead to Mutations in Children Leading to Acute Lymphoblastic Leukemia

NIEHS-funded research is investigating the connection of chemical exposures in parents and their children's risk of developing acute lymphoblastic leukemia (ALL). The epidemiologic study uses data from a large case-control study of childhood ALL conducted by the Children's Oncology Group in Southern California. The *ras* proto-oncogene family of genes has three members: H-*ras*, K-*ras*, and N-*ras*. *Ras* proto-oncogene mutations have been implicated in the development of many malignancies including pancreatic and breast cancers; however little data exists associating *ras* mutations with parental exposures and risk of childhood leukemia. DNA samples from the children in the study were examined for *ras* mutations. A total of 127 out of 837 ALL cases exhibited *ras* mutations in the K- or N-*ras* genes. A number of chemical exposures were associated with significantly increased risks for development of ALL in children. Parental use of "mind-altering drugs," such as marijuana, LSD, and cocaine was associated with increased risk for ALL (three-fold higher risk for maternal use and two-fold higher risk for paternal use). Maternal exposure to solvents and plastics during pregnancy raised the risk about three-fold and seven-fold, respectively, and maternal exposure to plastics after pregnancy was associated with eight times higher risk. Other exposures, such as to oil and coal products, were also associated with increased risks of ALL. In previous studies, parental occupational exposure to hydrocarbons such as chlorinated solvents, benzene, and paints has been linked to elevated childhood leukemia risk. The present study has extended these findings to include drugs of abuse and additional chemical exposures and to link them to *ras* mutations. The study's authors conclude that parental exposures to "hydrocarbons and mind-altering drugs, chemicals that have been previously suggested to increase the risk of childhood leukemia, [are] related to specific *ras* mutations in childhood ALL."

**Women Developing Myositis With and Without Silicone Implants Have Different Genetics**

Autoimmune diseases as now defined may consist of multiple distinct entities, each of which is characterized by different genetic and environmental exposures. For this reason, NIEHS clinical researchers compared women who developed myositis after silicone implants (MASI) with women developing myositis without implants (IIM) to determine if they differed in clinical, serologic, and/or genetic features. Two studies were undertaken—a case series followed by a larger, independent matched case-control study. In the case series study, 11 MASI patients differed from 76 IIM patients in having a significantly increased frequency of gene called HLA DQA1*0102 and decreased frequencies of the myositis-associated genes called DRB1*0301 and DQA1*0501. A subsequent matched case-control study revealed that while clinical features and autoantibodies did not differ significantly between MASI and IIM subjects, MASI patients again had decreased frequencies of DRB1*0301 and DQA1*0501 compared to IIM patients. Additional comparisons of MASI patients combined from both studies (n = 37) with a larger population of IIM patients (n = 453) suggested that the gene called HLA DQA1*0102 is uniquely associated with MASI. These studies suggest that women who develop inflammatory myopathy after silicone implants constitute a genetically distinct group of myositis patients. These findings support the growing belief that autoimmune diseases as we think of them today are actually heterogeneous collections of disorders defined by different genetic and environmental factors and suggests new avenues of investigation to dissect out the causes of these conditions.

**A Workshop Assessing Human Germ-cell Mutagenesis in the Post-genome Era**

NIEHS and the Office of Rare Diseases, with others, cosponsored the workshop "Assessing Human Germ Cell Mutagenesis in the Post-genome Era: A Celebration of the Legacy of William L. Russell." Russell was one of the pioneers in the field of mammalian germ cell mutagenesis. The workshop was held September 28–30, 2004, at The Jackson Laboratory in Bar Harbor, Maine, for the purpose of assessing current capabilities and projecting future strategies to detect human germ cell mutagens, identify genetic alterations, and evaluate long-term effects in human populations. Mutations in the human population can cause severe diseases and disability; while most are currently incurable, some can be treated with at least partial success. Collectively, they cause untold misery to parents and the affected offspring. The public and government have expressed concern about environmental agents that cause mutations, and many independent programs and international efforts have been funded over the years to study this problem. This workshop assessed whether recent progress in genomics could move the understanding of human germ cell mutagenesis forward. Attendees agreed that we must strive for better answers to the problems of human germ cell mutagenesis by using all the tools and resources available to us in this post-genomic era and that the effort needs to be multidisciplinary, including germ cell biology, genetic toxicology, bioinformatics, epidemiology, toxicogenomics, and other disciplines. Attendees reached consensus that an international program on human germ cell mutagenesis should be established before the end of calendar year 2005. The program should develop and evaluate parallel systems in humans and laboratory animals as well as develop efficient laboratory systems to identify agents that can induce transmissible mutations. The program should use the Human Genome Program as a model, drawing on lessons learned in that program to speed implementation and foster efficiency. Another component of the proposed program would be the establishment of a BioBank that would contain DNA and other biological specimens collected

from individuals exposed to suspect mutagenic agents and from those individuals' children, parents, and grandparents. These specimens would be accompanied by all available information on agents of exposure, dosages, personal profiles (protecting anonymity), and possible confounding factors. A proposed title for the overarching program in human germ cell mutagenesis is "The Etiology of Human Genetic Variation."

**A Workshop on Mold-related Health Effects**

The NIEHS and the NIH Office of Rare Diseases, with others, sponsored a workshop on "Mold-related Health Effects: Clinical, Remediation Worker Protection, and Biomedical Research Issues," June 28–29, 2004, at Washington Court Hotel in Washington, DC. The potential health effects related to exposure to mold and mold products in houses and workplaces have become a large public health concern. However, a lack of good clinical and experimental data is hampering evidence-based decision-making on how and when to conduct interventions, how to treat patients potentially harmed by mold exposure, how best to remediate affected buildings, and how to protect remediation workers. The workshop brought together experts in clinical science, worker protection and education, and basic research to further efforts to prevent, diagnose, and treat conditions related to exposure to indoor mold. The workshop consisted of an opening plenary session followed by two concurrent sessions, one dealing with clinical and biomedical research issues and the other with worker education and protection. Experts presented overviews of various areas along with their own data and some recommendations for future research. A closing plenary session presented overviews of the two concurrent sessions, and a final session presented clinical and research recommendations for future research. After the workshop, a list of the recommendations was compiled and emailed to participants, and they were asked to prioritize the recommendations.

This input has been received by workshop coordinators and is being evaluated, and a writer-contractor is preparing a summary of the meeting. This summary plus information on future research recommendations will be used to prepare a final report, which is scheduled for completion in March 2005.

## NATIONAL EYE INSTITUTE (NEI)

### Overview of Rare Diseases Research Activities

The National Eye Institute (NEI) was created on August 16, 1968, by Public Law 90-489 for the purpose of supporting and conducting research for improving the prevention, diagnosis, and treatment of diseases that affect the eye and vision. Eye diseases and blindness cost the Nation an estimated $38.4 billion per year. More than 12 million people in the United States suffer some significant impairment of vision. Over the years, vision researchers supported by the NEI have conducted many pioneering studies that have greatly advanced our understanding of eye diseases, including those classified as rare, and provided eye care professionals with new tools and methods to prevent or cure many sight-threatening conditions. In October 2003, the NEI released its strategic plan for vision research, *National Plan for Eye and Vision Research*. This plan is the seventh in the series that dates back to the publication of *Vision Research Program Planning* in 1975. The development and publication of the aforementioned plans address the visual health needs, including rare diseases of the eye and visual pathways, of the Nation.

### Recent Scientific Advances in Rare Disease Research

*Retinitis Pigmentosa and Related Disorders*

Retinitis pigmentosa (RP) is a group of blinding hereditary retinal degenerative diseases that are characterized by a progressive loss of vision due to the degeneration of photoreceptor cells. RP patients frequently report night blindness and loss of mid-peripheral vision during adolescence and are usually legally blind by the age of 40. Photoreceptor cells of the retina (the rods and cones) are responsible for the capture of light and the initiation of an electrical signal to the brain in the process of vision. The study of signaling in photoreceptor cells, termed the visual phototransduction cascade, has provided a detailed molecular description of this pathway.

*Adult bone marrow stem cells in retinitis pigmentosa*. A recent NEI-supported study found that injections of autologous adult bone marrow-derived stem cells prevented cone cell loss in two rodent models of RP. For reasons that are not entirely understood, the sick and dying rod cells also cause cone photoreceptor cells to die. Cone cells are concentrated in the macula, the center of the retina, and provide the sharp visual acuity that allows us to read, recognize faces, and perform detailed tasks that require hand-eye coordination. As the disease progresses patients lose their central vision, resulting in severe visual impairment or total blindness.

This study raises the possibility that patients could receive an injection of their own bone marrow stem cells to preserve central vision.

*Autoimmune Diseases*

*Bacterial toxin can prevent autoimmune uveitis.* Human autoimmune uveitis comprises a group of inflammatory eye diseases affecting the interior of the eye that can severely compromise vision. Autoimmune uveitis is thought to be caused by an immune response to proteins in the retina of the eye. Current therapies consist of immunosuppressive drugs such as steroids, cyclosporin,

antimetabolites, and alkylating agents, all of which have severe side effects. NEI-intramural scientists are using an animal model, experimental autoimmune uveitis (EAU) in laboratory mice, to study the disease and to develop novel therapies based on reprogramming the immune system (immunomodulation) rather than on immunosuppression using pharmacologic agents. Bacterial components tend to have strong effects on the immune response. The class of compounds known as bacterial toxins can alter immune responses when included in small nontoxic doses in an immunization protocol. Cholera toxin has been studied as an additive to mucosal vaccines and found to promote a particular type of response having a unique immune mediator profile known as Th2. In contrast, EAU and similar autoimmune diseases develop in the context of an immune response having a mediator profile known as Th1. Th1 and Th2 responses are mutually inhibitory. Promoting a Th2 response may inhibit the Th1 response involved in uveitis. These researchers are exploring the ability of cholera toxin to counteract the Th1 response leading to development of EAU in mice. They have found that a single injection of as little as 2 micrograms of cholera toxin (a harmless dose) into mice immunized at the same time with a uveitogenic regimen of the retinal antigen IRBP completely protected the mice from developing this blinding disease. The protected mice were not immunosuppressed but rather displayed evidence of immune deviation toward a Th2-like immune response. Further studies of the mechanisms involved showed that cholera toxin caused production of an immune mediator known as IL-4, which is known to promote Th2 responses. This mediator was responsible for diverting the immune response toward the harmless Th2 type, thereby replacing the harmful Th1 response and preventing disease. These data suggest that, in general, immunomodulatory treatments that skew the immune response toward the Th2 pathway may be beneficial in treating ocular autoimmune disease. It is important to note that in this animal model disease is prevented but not reversed; this is a valuable treatment paradigm, because chronic autoimmune disease depends on continuous recruitment of new autoreactive lymphocytes. If these new recruits can be redirected toward the harmless Th2 pathway, progression of disease will be interrupted.

**Rare Disease-related Program Activities**

The National Advisory Eye Council and the NEI have established the following goals for rare disease research in the *National Plan for Eye and Vision Research*.

$ Understand the pathogenesis of inherited retinal diseases.
$ Continue to develop models and a coordinated system to share animal model data and resources in the vision community.
$ Characterize the genes and proteins expressed in tissues of the ocular surface; determine the functional consequences of changes in expression and molecular interactions; and determine the epigenetic, hormonal, neural, and environmental influences under both normal and pathological conditions.

**Rare Disease-related Program Scientific Conferences, Workshops, Symposium, and Meetings**

On November 5, 2004, the NEI cosponsored a 2-day symposium in Washington, DC, entitled *The First International Symposium on Translational Clinical Research for Inherited and Orphan Retinal Diseases*. The other cosponsors included: National Neurovision Research Institute, Inc;

Foundation Fighting Blindness; Office of Rare Diseases, NIH; National Institute on Aging; National Institute of Neurological Disorders and Stroke; National Heart, Lung, and Blood Institute; National Institute on Deafness and Other Communication Disorders; Office of Orphan Products Development, Food and Drug Administration; Alcon; and W. K. Kellogg Foundation.

The objectives of the symposium were to:

- Promote drug and genetic translational clinical research for orphan retinal disease therapy.
- Review status, case studies, and challenges of inherited orphan disease research.
- Facilitate interaction across vision, neurology, gerontology, and audiology.
- Define the strategies and dynamics for commercializing orphan disease treatments.
- Prioritize and develop strategies for intellectual property, operational, and financial avenues to bring new therapies to patients.

## NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES (NIGMS)

### Overview of Rare Diseases Research Activities

The National Institute of General Medical Sciences (NIGMS) supports broad-based fundamental research that is not targeted to any specific organ system or disease. Examples include studies on the structure and function of organelles and membranes at the cellular and molecular level; investigations into the replication, organization, and function of the genome in organisms ranging from bacteria to man; development of new and improved instrumentation and technology for application to biological problems; studies on basic bio-related organic chemistry for the elucidation of biosynthetic pathways and the development of new synthetic strategies for molecules of biological interest; and investigations of basic pharmacological mechanisms at levels ranging from the receptor to the molecular. In general, support of investigations related to specific diseases, unless of wide applicability across disease or organ system lines, is not the responsibility of the NIGMS but rather would be assigned to one of the categorical Institutes.

*Human Genetic Cell Repository*

The NIGMS Human Genetic Cell Repository provides a valuable resource for investigators studying genetic disorders. The Repository, located at the Coriell Institute for Medical Research in Camden, NJ, collects, characterizes, maintains, and distributes cell lines and DNA samples from patients and families with a wide variety of genetic disorders and from normal persons whose tissues serve as controls. More than 9,200 unique cell lines, representing over 700 different diseases, and 4,000 DNA samples are available to qualified investigators. The Repository stimulates research on rare diseases by providing access to cell lines, both fibroblasts and transformed lymphoblasts, and DNA samples derived from these cell lines, that otherwise are not readily available. Among the cell lines requested most frequently in the last year are those from patients with rare diseases such as xeroderma pigmentosum, ataxia-telangiectasia, Fanconi anemia, cystic fibrosis, fragile X-linked mental retardation, Niemann-Pick disease, Friedreich ataxia, familial breast cancer, osteogenesis imperfecta, and Down syndrome. Recent acquisitions to the collection include samples from patients with the following rare disorders: adrenoleukodystrophy, Alexander disease, Alpers syndrome, ATP synthase deficiency, Caravan disease, Ehlers-Danlos syndrome (Types III, IV, unclassified), fascioscapulohumeral muscular dystrophy, RETT syndrome, osteogenesis imperfecta, hypochondroplasia, glycogen storage disease types VIII and IX, and Salla disease. These cell lines, as well as those previously acquired, are used for biochemical, cellular, and molecular studies to help elucidate the causes of genetic defects. The Repository has a growing collection of cell lines in which the mutation has been characterized at the molecular level. These include the newly acquired samples from patients with adrenoleukodystrophy, Alexander disease, Bloom syndrome, galactosemia, and osteogenesis imperfecta as well cell lines with recently characterized mutations from patients with cystinuria, familial dysautonomia, and multiple Nieman-Pick C variants.

In addition, the Repository houses an expanding collection of chromosomally aberrant human cell lines. It also supplies DNA isolated from complete panels of well-characterized human-rodent somatic cell hybrids and from chromosome-specific somatic cell hybrid panels for nearly every human chromosome.

The Repository also houses sets of cell lines (and DNAs derived from them) that represent the CEPH family collection and other extended families, the National Human Genome Research Institute's DNA Polymorphism Discovery Resource and International HapMap Project, and other identified populations that represent the genetic diversity of humans. These samples will help researchers map and identify genes that are involved in the etiology of complex genetic diseases.

**Recent Scientific Advances in Rare Diseases Research**

*Unstable DNA Repeats in Neurodegenerative Disorders*

Over a dozen inherited neuromuscular degenerative diseases, including Huntington's disease (HD), myotonic dystrophy, Friedreich's ataxia, and fragile X syndrome, are associated with the presence of long tracts of trinucleotide repeats (TNRs) in genomic DNA. There are normally fewer than 30 repeats in any particular chromosomal allele, but affected individuals may have several hundred repeat units at a disease-causing gene locus. A process by which the length of TNR tracts may rapidly expand to several hundred copies in a single generation was addressed in work supported by NIGMS that was reported last year. Now, another group of NIGMS-supported researchers has reported their insights into the details of the pathogenic mechanisms involving these repeat structures that can not only compromise chromosomal integrity but also lead to immediate cell death in these human diseases.

The investigators initially observed, using a yeast model system, that just the presence of TNRs in growing cells is associated with activation of the DNA damage cell cycle checkpoint response. It appears that the unusual DNA structures that can arise during the replication of triplet repeats, particularly long repeat tracts, are seen as chromosome defects by the DNA damage control monitor. If these apparent lesions are not repaired quickly and efficiently, the activated cell cycle checkpoint machinery triggers the programmed cell death (apoptosis) pathway. The mechanisms of this cellular response, which serves to prevent the reproduction of cells with chromosomal defects, are conserved in yeast and humans. Overactivation of the checkpoint mechanism, in cells that are already stressed to repair multiple possible defects, represents an obvious explanation for the death of neurons and muscle cells in HD and myotonic dystrophy and other TNR diseases. Future work to characterize checkpoint mechanisms may provide approaches for limiting the severity of TNR expansions and the manifestation of the associated pathologies.

*Polyglutamine Protein Inhibition of Proteosomes in Neurodegenerative Diseases*

The long tracts of TNRs that are present in the chromosomal DNA sequences of patients suffering from hereditary neurodegenerative disorders can compromise cell function through a spectrum of pathogenic mechanisms ranging from the disruption of chromosome replication to the cytoplasmic accumulation of massive, toxic aggregates of defective proteins. HD and spinocerebellar ataxia (SCA) are associated with expansion of CAG nucleotide repeats in coding regions of the genes coding for the huntingtin and ataxin-1 kinase proteins, respectively. Transcription and translation of the CAG codons results in the incorporation of long polyglutamine stretches in these peptides. NIGMS-funded researchers have confirmed that the toxic protein clumps observed in cells from these patients originate initially as result of the self-aggregation of polyglutamine containing

huntingtin and ataxin-1 proteins, but they add that the aggregation is exacerbated as the toxic protein complexes irreversibly bind to and inhibit the function of proteosomal.

Proteasomes are the molecular machines that are responsible for the normal turnover of proteins that is central to cell homeostasis. In healthy cells, proteins perform their various functions and then, through the action of the proteasome, are degraded and cleared. The new results indicate that in HD, SCA, and other polyglutamine diseases, the inhibition of these machines not only prevents the complete degradation of the polyglutamine peptides but also impairs the normal degradation of other aged and damaged proteins that then become entangled in the expanding aggregates. A detailed understanding of the distinct biophysical properties and molecular interactions that result in differences in the rate of complex formation and in the degree of proteosomal compromise with huntingin and ataxin-1 may enable researchers to account for differing presentation of the disease processes. This basic knowledge could lead to interventions for these currently untreatable conditions.

## NATIONAL HEART, LUNG, AND BLOOD INSTITUTE (NHLBI)

### Overview of Rare Diseases Research Activities

The National Heart, Lung, and Blood Institute (NHLBI) provides leadership for a national program in the causes, diagnosis, treatment, and prevention of diseases of the heart, blood vessels, lungs, and blood; in sleep disorders; and in the uses of blood and the management of blood resources. Through research in its own intramural laboratories and through extramural research grants and contracts, it conducts and supports an integrated program that includes basic research, clinical trials, epidemiological studies, and demonstration and education projects.

Although the major part of the research supported by the NHLBI addresses common conditions such as hypertension, coronary heart disease, and chronic obstructive pulmonary disease, a significant amount of research is devoted to rare diseases in children and adults. NHLBI activities related to rare disease research in fiscal year (FY) 2004 are described below.

### Recent Scientific Advances in Rare Diseases Research

### Heart and Vascular Diseases Programs

*Abetalipoproteinemia*

Abetalipoproteinemia is a recessive disorder of lipid metabolism characterized by the absence of apoprotein B-containing lipoproteins from the plasma. Symptoms include diarrhea, severe fat malabsorption, and acanthocytosis, a rare condition in which the majority of red blood cells have multiple spiny cytoplasmic projections. Other symptoms, including blindness and neurologic defects, all appear to be secondary to defects in the transport of vitamins A and E in the blood. The disorder is associated with abnormal processing of apolipoprotein B (apo B), which is caused by the absence of microsomal triglyceride transfer protein (MTP). Researchers in the NHLBI intramural program are studying patients with abetalipoproteinemia to document the natural history of the disorder and to look for more effective treatments. They also are conducting metabolic studies in patients with abetalipoproteinemia to clarify the role of MTP in triglyceride (fat) and fat soluble vitamin absorption. NHLBI-supported extramural researchers are actively investigating MTP's role in the formation of apo B-containing lipoproteins. Their efforts include studies with hepatoma cells that lack MTP and that are therefore incapable of assembling or secreting apo B-containing lipoproteins. In addition, researchers have recently developed a new method for measuring MTP.

*Antiphospholipid Syndrome*

Antiphospholipid syndrome (APS) symptoms include recurrent blood clotting disorders, a history of fetal deaths, and autoimmune diseases such as thrombocytopenia. APS also increases the risk of developing atherosclerosis. Many patients with the disorder also have systemic lupus erythematosus (SLE). APS is characterized by the presence of circulating autoantibodies to certain phospholipid-binding proteins such as $B_2$-glycoprotein I (also known as apolipoprotein H). The NHLBI supports research to determine whether genetic factors predispose individuals to develop

antibodies. Researchers also are developing more standardized immunoassays for reliable detection of antibodies. In addition, investigators are studying the role of the autoantibodies in atherogenesis.

*Arrhythmogenic Right Ventricular Dysplasia*

Arrhythmogenic right ventricular dysplasia (ARVD) is a family of rare cardiomyopathies that result in abnormal heart rhythm and sudden cardiac death. Most forms are believed to be caused by the inheritance of autosomal dominant mutations in genes that remain largely unknown but clearly affect myocardial integrity. ARVD is characterized by marked, selective, right ventricular dilatation, myocardial cell death, and cell replacement with fat cells and fibrous tissue. Expression in gene carriers is variable but, in those who display symptoms, the disease is frequently fatal. The NHLBI supports the Multidisciplinary Study of Right Ventricular Dysplasia, an integrated network of three separate groups, to investigate genotype-phenotype relationships in familial forms of ARVD. The program uses clinical and genetic approaches to identify patients with ARVD. Study investigators are performing linkage analysis and candidate positional cloning to identify genetic regions and plausible candidate genes of interest. Two studies recently added to the Multidisciplinary Study of Right Ventricular Dysplasia should improve diagnosis and characterization of ARVD patients. An FY 2004 publication from Multidisciplinary Study of Right Ventricular Dysplasia investigators reported that (1) genetic heterogeneity exists in arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), (2) mutations in the Desmoplakin gene are a relatively common cause of ARVD/C, (3) a potential genetic locus for ARVD/C is found on chromosome 8p23.2, and (4) the probable 'final common pathway' of ARVD/C involves cellular structures called adherens junctions. Another recent ARVD-related study from NHLBI-funded investigators reported that the high frequency of misdiagnosis of ARDV/C is due in part to over-reliance on the presence of fat and wall thinning on magnetic resonance imaging (MRI) tests and incomplete diagnostic testing. The study investigators concluded that diagnosis of ARDV/C should not rely solely on qualitative results of MRI testing.

*Bartter's Syndrome*

Bartter's syndrome is characterized by salt imbalance and low blood pressure. Research on Bartter's syndrome is currently being pursued as a part of the NHLBI Specialized Centers of Research on Molecular Genetics of Hypertension. Researchers have discovered a mutation in a potassium channel that can lead to Bartter's syndrome and have demonstrated that the channel is an important regulator of blood pressure and ion and fluid balance. Mutations in chloride channels have also been identified and implicated in the development of Bartter's syndrome. Researchers think that additional Bartter's syndrome genes may exist. The hypotensive state caused by Bartter's syndrome suggests that the mutated genes may protect against the development of high blood pressure.

*Beta-sitosterolemia*

Beta-sitosterolemia is a rare inborn error of metabolism characterized by increased absorption of dietary cholesterol and sterols from plants and shellfish. The distinguishing feature of the disorder, a 50- to 100-fold elevation in plasma plant sterol levels, reflects both an increase in absorption of

sterols from the intestine and a decrease in excretion of sterols into bile. People with beta-sitosterolemia have a markedly increased risk of premature cardiovascular disease. Effective treatment is not available at present, although a number of drugs are under development. Recent evidence suggests that sitosterolemic patients have mutations in either the ATP-binding cassette half-transporter (ABCG5) gene or in ABCG8, a similar gene that resides next to it in the genome. The phenotype resulting from deficiency of the ABCG5 protein (i.e., sitosterolemia) is identical to that caused by deficiency of ABCG8 protein, indicating that the two proteins participate in the same transport process. The profound alteration in sterol homeostasis observed in sitosterolemic patients indicates that ABCG5 and ABCG8 play a pivotal role in sterol metabolism. Researchers in the NHLBI's intramural program are conducting molecular, cellular, and metabolic studies of beta-sitosterolemia. They are also investigating the use of a new cholesterol absorption inhibitor for the treatment of beta-sitosterolemia. Researchers funded by NHLBI extramural research grants are studying patients with sitosterolemia and other mutations of sterol absorption, basic mechanisms of sterol absorption, and related disorders of sterol metabolism.

*Brugada's Syndrome*

Brugada's syndrome, a rare inherited disorder characterized by cardiac electrophysiological abnormalities (right bundle branch block and ST elevation in the precordial leads), is associated with a high occurrence of sudden cardiac death (SCD). The condition is currently believed to be similar in cause and potential treatment to some forms of Long QT syndrome, which is caused by mutations at different locations in the SCN5A cardiac muscle sodium ion channel gene. The NHLBI supports a Program Project Grant and a small portfolio of individual research project grants to study the molecular and genetic bases of Brugada's syndrome. NHLBI-funded investigators have shown that electrocardiographic testing, combined with the use of the sodium channel blocker ajmaline, can identify nonsymptomatic carriers of defective sodium channel genes in affected families. The complex and challenging nature of Brugada's syndrome research was evidenced in a separate study when research on a large family with a high prevalence of SCD showed that ajmaline can sometimes provide false-positive results.

*Cholesteryl Ester Storage Disease*

Cholesteryl ester storage disease (CESD) is a rare syndrome characterized by an enlarged liver and spleen and abnormal liver enzymes. The disorder is known by a variety of names including lysosomal acid lipase deficiency, acid cholesteryl ester hydrolase deficiency, and Wolman disease. The severe infantile-onset Wolman disease and the milder late-onset CESD are seemingly caused by mutations in different parts of the acid lysosomal lipase gene. The mutations affect acid lysosomal lipase, an enzyme that removes lipids (cholesteryl ester and triglycerides) from lipoproteins. Acid lysosomal lipase deficiency causes a massive accumulation of lipids in tissues. The hypercholesterolemia that is common in individuals with CESD predisposes them to develop atherosclerosis. Researchers in the NHLBI's intramural program are conducting studies to determine the role of acid lysosomal lipase in the removal of lipids from the plasma and from tissues.

*Congenital Heart Defects*

Congenital heart defects encompass a constellation of abnormalities in the heart that occur during embryonic development. Abnormalities of the heart are the most common of birth defects, occurring in up to 1 percent of live births, and they are an important cause of infant mortality, pediatric and adult morbidity, and shortened adult life expectancy. About one-third of affected infants and children require open heart surgery or interventional cardiac catheterization to repair or ameliorate their defects. Approximately the same proportion has associated extracardiac anomalies, such as chromosomal abnormalities and syndromes involving other organ systems.

The NHLBI has supported research in pediatric cardiovascular medicine since it first funded heart research grants in 1949. NHLBI-supported researchers have been instrumental in developing diagnostic fetal imaging techniques, surgical techniques, and medical therapies now used to ensure healthy survival for most affected children. They also have made significant contributions to the epidemiology of congenital heart defects and to understanding the molecular and genetic basis of normal and abnormal heart development. Recently, NHLBI-supported researchers have investigated the mechanism by which mutations in the Nkx2.5 gene generate conduction defects in congenital heart defects. Furthermore, they have been able to relate their work in animal models to human disease by confirming that humans with an Nkx2.5 mutation show the same defects in cardiac conduction system development as are observed in mutant mice. The NHLBI Division of Intramural Research is studying the genetics of congenital heart defects by generating new mutations in mice and then using high-frequency ultrasound to screen the offspring for congenital heart defects during the fetal period. Several gene mutations have been identified that replicate forms of congenital heart defects observed in human patients. A recipient of an NHLBI Mentored Minority Faculty Development Award showed that, in mice, embryonic stem cells can prevent cardiac malformations (that otherwise would have been caused by a genetic mutation) due to production of growth factor proteins that affect cardiac development. Further investigation of these factors may lead to new treatments to prevent or reduce the severity of congenital heart defects.

*DiGeorge Syndrome*

DiGeorge syndrome occurs in about 1 in 4,000 live births. The syndrome causes many abnormalities, including cardiac outflow tract anomalies, hypoplasia of the thymus and parathyroid glands, cleft palate, facial dysmorphogenesis, learning difficulties, and other neurodevelopmental deficits. It is usually sporadic, but may be inherited, and is caused by deletion of a segment of chromosome 22, which is known to contain numerous genes. The recent identification of a gene responsible for much of DiGeorge syndrome has enabled further research on how the gene is regulated and how it affects embryonic development through regulation of downstream genes. In FY 2004, researchers reported that differences in regulation of the gene in the heart, compared to its regulation in other organs affected by DiGeorge syndrome, explain why heart malformations are more common in patients than are certain craniofacial defects. The NHLBI funds grants for both human and animal studies of DiGeorge syndrome, including newly funded Specialized Centers of Clinically Oriented Research (SCCOR) in Pediatric Heart Development and Disease. Much of the NHLBI-supported basic research in congenital heart defects also enhances

understanding of DiGeorge syndrome because several of the most frequent cardiac malformations occur in conjunction with the disorder.

*Dysbetalipoproteinemia*

Dysbetalipoproteinemia, or type III hyperlipidemia, is a disorder with a strong heritable component characterized by the presence of beta-migrating very low density lipoprotein (VLDL). Dysbetalipoproteinemia leads to the formation of characteristic yellow skin plaques (xanthomas) and predisposes to premature ischemic heart disease and peripheral vascular disease. The defect occurs in people with mutated forms of a protein, apoprotein E (apo E). A mutant form of apo E, apo E2, has been identified as the chief molecular defect. Animal models have been developed to facilitate basic research on this disease. Mice expressing human apo E2 show increased plasma cholesterol and triglyceride levels. Similar increases in lipid levels are seen in humans with type III hyperlipidemia. Hyperlipidemia can be ameliorated in the mice by increasing expression of the low-density lipoprotein receptor by two-fold. The NHLBI Division of Intramural Research is investigating the relationship between mutant apo E and the development of dysbetalipoproteinemia. Intramural researchers are studying patients with the disease to define its natural history and develop better treatments.

*Familial Hypobetalipoproteinemia*

Familial hypobetalipoproteinemia (FHBL) is a disorder of lipid metabolism characterized by greatly reduced levels of apoprotein B (apo B)-containing lipoprotein cholesterol. Several different types of FHBL have been found, each resulting from a different mutation. Scientists estimate that fatty livers (which have a five-fold increase in fat compared to normal livers) may be present in up to 80 percent of people with a form of FHBL resulting from apo B truncation. Individuals with the most severe form of the disease develop defects in the transport of vitamins A and E in the blood, resulting in blindness and neurologic defects. In one group of patients with FHBL, a gene mutation on chromosome 2 has been identified and characterized. The mutation results in truncation of apo B. Another group of eight families has been identified that appear to have a different form of FHBL with involvement of a different region of chromosome 2. A genome-wide search suggests another candidate gene on chromosome 3. Mechanistic studies infer that the fatty livers found in patients with apo B-defective FHBL are caused by an inability of the liver to export triglyceride. The NHLBI intramural program is investigating the role of apo B and other factors in the development of FHBL. Intramural researchers are studying patients with FHBL to clarify the natural history of the disorder and to look for more effective treatments. They also are performing metabolic studies in patients to clarify the role of apo B and other agents that might cause defects in absorption of triglycerides (fat) and fat soluble vitamins. The NHLBI extramural program funds research grants to study genetic, biochemical, and metabolic aspects of FHBL.

*Homozygous Familial Hypercholesterolemia*

Familial hypercholesterolemia (FH) is an inherited disorder characterized by elevated concentrations of low-density lipoproteins (LDL). The homozygous form of FH is rare (one in a million), but people who have it are very prone to premature coronary heart disease.

Cholesterol derived from LDL, when deposited in arteries, leads to heart attacks and, when deposited in tendons and skin, causes xanthomas. FH is caused by a mutation in a gene specifying the receptor for plasma LDL. LDL receptors facilitate the removal of LDL. When they are deficient or absent, the rate of LDL removal declines, causing the level of LDL in the plasma to rise. The NHLBI Division of Intramural Research is investigating the role of the LDL receptor in FH. Patients in intramural studies are eligible to undergo serial testing that includes noninvasive cardiac monitoring and measurement of biomarkers. The goal of the studies is to document the natural history of FH and the development of atherosclerosis. Recently, intramural researchers have made progress in the use of MRI for noninvasive evaluation of the development of aortic root atherosclerosis in patients with familial hypercholesterolemia. In the NHLBI extramural program, several grants support studies on the biochemistry, genetics, and potential treatment of the disease.

*Lecithin Cholesterol Acyltransferase Deficiency*

Lecithin cholesterol acyltransferase (LCAT) deficiency is a rare syndrome characterized by cloudy cornea, kidney failure, and extremely low levels of high-density lipoprotein (HDL). The disorder is inherited on chromosome 16 and is caused by a lack of the enzyme LCAT, which aids in the formation of normal HDL. Researchers in the NHLBI's intramural program are investigating molecular, cellular, and metabolic defects associated with human LCAT and their role in LCAT deficiency. Patients with LCAT deficiency are being studied to clarify the natural history of the disorder and to develop better treatments. Intramural researchers are also performing studies in patients to determine the role of LCAT in HDL formation and in the development of atherosclerosis.

*Lipoprotein Lipase Deficiency*

Lipoprotein lipase deficiency (LPL) is a rare genetic lipid disorder characterized by extremely elevated triglyceride (fat). It is caused by a genetic defect that affects an enzyme involved in breaking down triglyceride (fat) and removing it from the blood. The specific gene defect, which is found on chromosome 8, is in the enzyme LPL gene. The excess triglycerides characteristic of the disorder are often deposited in the skin (eruptive xanthomas), back of the eye (lipemia retinalis), liver, and spleen and cause abdominal pain or pancreatitis in children. Researchers in the NHLBI intramural research program are conducting metabolic studies in patients with LPL deficiency to determine the role of the lipases in the metabolism of triglyceride lipoproteins and in the development of atherosclerosis and pancreatitis.

*Klippel-Trenaunay-Weber Syndrome*

Klippel-Trenaunay-Weber syndrome (KTWS) is a very rare, vascular deformation disease of unknown incidence, involving capillary, lymphatic, and venous channels. It usually manifests itself as three symptoms: cutaneous port-wine capillary malformations, varicose veins, and enlargement of soft tissues and bone in one limb. KTWS symptoms are usually present at birth, with 75 percent of patients having symptoms before the age of 10. One NHLBI grant supports molecular research on characterizing the gene(s) responsible for KTWS.

Recently, researchers found a *de novo* supernumerary small ring marker chromosome derived from chromosome 18 in a patient with Klippel-Trenaunay syndrome, the first demonstration of such an extra chromosome in a patient with KTWS. The finding is unique for a rare vascular-overgrowth syndrome and may help researchers to define candidate genes for this disorder.

*Liddle's Syndrome*

Liddle's syndrome is a disorder of severe hypertension, characterized by increased renal reabsorption of sodium resulting in hyperaldosteronism, or overproduction of the hormone aldosterone from the outer portion (cortex) of the adrenal gland. The hyperaldosteronism results in low potassium levels (hypokalemia), reduced acidity of the body (alkalosis), muscle weakness, excessive thirst (polydipsia), increased urination (polyuria), and hypertension. Research on Liddle's syndrome is currently being pursued as part of the NHLBI's Specialized Centers of Research on Molecular Genetics of Hypertension.

*Long QT Syndrome*

Long QT syndrome (LQTS) is characterized by a prolonged QT segment on an electrocardiograph and is associated with fainting (syncope), ventricular arrhythmias, and, frequently, sudden cardiac death. LQTS comprises a family of related diseases that are often inherited and that are repolarization induced by mutations in cardiac ion channel genes. In some forms of the disease, affected individuals may inherit other abnormalities, such as deafness, and have varied clinical outcomes depending on their specific mutational patterns. About 70 percent of diagnosed cases are in women. Recently, three specific gene mutations have been identified (KvLQT1, HERG, and SCN5A), which are associated with three forms of LQTS (LQT1, LQT2, and LQT3). The NHLBI currently supports research on LQTS through a Specialized Center of Research on Sudden Cardiac Death and through many other grants that address the molecular, clinical, and genetic bases of this condition. The NHLBI also supports an investigator-initiated grant comprising an international LQTS registry with over 1,000 families. It includes 2,235 affected individuals, 1,275 family members with borderline LQTS, and 2,429 unaffected family members. Investigators are continuing genetic investigations to identify known genetic variants in registry members and to identify new variants and mutations. LQTS researchers also are assessing genotype-phenotype correlations as well as the long-term course of the disease by evaluating triggering factors for malignant ventricular arrhythmias.

Several recent advances in LQTS research will help guide the clinical care of LQTS patients. Some recent findings are that (1) age and gender have different, genotype-specific modulating effects on the probability of cardiac events and electrocardiographic presentation in LQT1 and LQT2 patients (2) LQT2 patients with mutations in the pore region of the HERG gene are at increased risk of arrhythmic events compared to those whose mutations are in the nonpore regions (3) exercise triggers adverse events in LQT1 patients, whereas most events in LQT2 and LQT3 patients occur during rest or sleep (4) unexpectedly common mutations in five of the genes responsible for cardiac potassium and sodium ion channel regulation are related to the expression of congenital LQTS in certain families, and (5) individuals with atypical LQTS have a defective gene coding for production of a defective cardiac cell structural protein believed to cause sodium, potassium, and calcium imbalances; unusually long QT intervals; arrhythmias; and sudden death.

In addition, studies of nervous system involvement in LQTS have led to new types of successful surgical management of high-risk patients and to a new oral therapy for one type of LQTS.

*Marfan Syndrome*

Marfan syndrome is an inherited connective tissue disorder associated with cardiovascular complications such as aortic aneurysms (which are potentially fatal) and mitral valve prolapse as well as noncardiac complications such as dislocation of the eye lens. The disease occurs in about 1 per 10,000 persons and in all races. The NHLBI supports animal research on the assembly of microfibrils and their effects on cardiovascular development as well as on aortic aneurysm development and its treatment in the atherosclerotic population. The research may have implications for treating aneurysms in people with Marfan syndrome.

The NHLBI plans to support a data coordinating center to develop and maintain a registry of patients with Marfan syndrome and other connective tissue diseases who receive treatment for cardiovascular complications and to enable research to determine best medical practices for advancing awareness and clinical management of genetic thoracic aortic aneurysms and other cardiovascular complications. The registry will: (1) collect information on patients, care providers, hospitals, and clinical interventions; (2) collect blood and tissue specimens; and (3) maintain a repository of tissue and blood, family pedigrees, and data on extra-cardiac complications. The NHLBI will collaborate with the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Eye Institute (NEI), the National Institute of Dental and Craniofacial Research (NIDCR), and the National Human Genome Research Institute (NHGRI) in facilitating standardized reporting by the registry of patient characteristics, indications for surgical intervention and other treatments, and adverse events. The National Marfan Foundation, which participated in a 2002 NHLBI-sponsored working group on this disorder, is fully supportive of the registry and intends to encourage participation from its membership.

*Niemann-Pick Type C Disease*

There are several types of Niemann-Pick (NP) disease: types A (NPA), B (NPB), C (NPC), and D (NPD). NPC disease is a lipid storage disorder usually characterized by excessive accumulation of cholesterol in the liver, spleen, and other vital organs. Affected individuals have cardiovascular disease, enlargement of the liver and spleen (hepatosplenomegaly), and severe progressive neurological dysfunction. The gene deficiency in NP disease types A and B affects sphingomyelinase, an enzyme that breaks down the lipid sphingomyelin. The gene deficiency in NP disease types C and D affects the NPC-1 protein whose function remains obscure. Animal studies and basic research show that mutations in NPC-1 interfere with lipid metabolism, cholesterol homeostasis, and intracellular cholesterol trafficking. Although the dysfunctions cause severe damage to the nervous system, bone marrow, and other tissues and organs in people with NPC, they also appear to stabilize atherosclerotic plaques against rupture and may thus protect adults who carry the NPC mutation from cardiovascular events such as heart attack, angina, and stroke. The defect in intracellular cholesterol movement leads to abnormal accumulation of cholesterol in a cellular compartment called the lysosome.

The NHLBI funds grants for research on regulation of intracellular cholesterol movement that leads to lipid accumulation in NPC disease.

*Smith-Lemli-Opitz Syndrome*

Smith-Lemli-Opitz syndrome (SLOS) is an inherited disorder caused by a defect in an enzyme active in the last step of cholesterol biosynthesis. As a result of the defect, endogenous cholesterol synthesis is inadequate for meeting biological demands for functions such as membrane structure and bile acid synthesis. The disorder also leads to accumulation of the cholesterol precursor 7-dehydrocholesterol and its derivatives. Newborns with SLOS have a distinctive facial dysmorphism; suffer from multiple congenital anomalies including cleft palate, congenital heart disease, genitourinary abnormalities, and malformed limbs; and exhibit digestive difficulties, severe developmental delays, and behavioral problems. Scientists now think that SLOS could be the cause of many previously unexplained cases of mental retardation. During FY 2004, the NHLBI supported two investigator-initiated grants on SLOS. One investigator is using transgenic animal models of SLOS to study the basic pathophysiology of the condition, to develop improved molecular therapeutic approaches, and to increase understanding of the normal role of cholesterol in fetal development. The other researcher is investigating sterol balance and lipid metabolism in infants with SLOS, the effectiveness of cholesterol-supplemented baby formula in ameliorating some of the behavioral and digestive symptoms of SLOS, and the effectiveness of simvastatin therapy in lowering the plasma concentrations of toxic forms of abnormal cholesterol precursor compounds. In FY 2004, investigators reported that providing additional dietary cholesterol as egg yolk to SLO infants successfully raises plasma lipid levels in favorable ways. The functional implications of the finding are not yet clear because certain measures of developmental progress do not appear to change in response to dietary cholesterol. Therefore, the interesting findings do not yet form a basis for altering clinical management for children with SLOS. Future research activities will explore the effects of different types of dietary manipulations in detail.

*Supravalvular Aortic Stenosis*

Supravalvular aortic stenosis (SVAS) is a vascular proliferative obstructive disease that affects the aorta and the coronary, carotid, and peripheral arteries. The incidence of SVAS is thought to be less than 5 percent of all congenital heart defects. Researchers think that SVAS is caused by a mutation in the gene for elastin, an extracellular matrix protein accounting for about 50 percent of the dry weight of the vascular wall. Recent studies suggest that native elastin maintains the contractile phenotype of vascular smooth muscle cells. The mutation in elastin found in individuals with SVAS activates genes that contribute to a change in the phenotype of vascular smooth muscle cells from a contractile nonmigratory state to noncontractile, migratory, proliferating state. The NHLBI supports three grants focused on SVAS.

*Tangier Disease*

Tangier disease is a rare syndrome characterized by a deficiency of high-density lipoprotein (HDL), mild hypertriglyceridemia, neurologic abnormalities, and massive cholesterol ester deposits in various tissues such as the tonsils.

The disease is inherited and appears to be caused by excessive breakdown of HDL rather than by a fault in HDL synthesis. Tangier disease patients have defective intracellular lipid trafficking that prevents removal of cholesterol from cells. A member of the ATP-binding cassette (ABC) transporter family (human ABCA1) located on chromosome 9 has been identified as the defective gene in Tangier disease. ABCA1 is thought to be the gatekeeper for eliminating excess cholesterol from tissues and, therefore, key in determining cholesterol accumulation in arterial walls. Researchers in the NHLBI's intramural program are studying patients with Tangier disease to clarify its natural history. Their research has shown an intracellular role for ABCA1 in the removal of cholesterol from peripheral cells. They also have shown that ABCA1 is involved in the formation of HDL particles from the liver. Metabolic studies performed by intramural researchers are helping to expand understanding of the role of the different HDL particles in the removal of cholesterol from the body. The NHLBI also funds extramural research grants to investigate the cell biology and biochemistry of human ABCA1 and its role in Tangier disease. Recent research efforts have been directed at understanding the critical regions in apo AI necessary for ABCA1-mediated lipid efflux. Families with low HDL who have new variants of ABCA1 have been identified and the functional significance of their variants is being examined.

*Trimethylaminuria*

Trimethylaminuria (TMAU) is caused, most often, by genetic mutations that inactivate specific liver enzymes, leading to defects in the body's ability to break down trimethylamines (TMAs). TMAs are volatile compounds that are produced by the action of gastrointestinal bacteria on choline and related substances derived from the diet. TMAU occasionally occurs with some liver and kidney diseases and with other genetic syndromes such as Prader-Willi syndrome. In people with TMAU, excess TMA is excreted in sweat and exhaled into the air causing offensive odors that lead to severe social isolation. Some patients experience constant symptoms and others have fluctuations in the intensity of odors. Neurologic and psychiatric symptoms, such as seizures and depression, may also be present. Most cases are diagnosed in adults, although onset in childhood can occur. Diagnosis is difficult and compromised by social stigma. Case reports suggest that symptoms in many TMAU patients can be ameliorated by diets low in choline, lecithin, carnitine, lysine, other dietary amines, and vegetables from the cabbage family.

**Lung Diseases Programs**

*Advanced Sleep Phase Syndrome*

Advanced sleep phase syndrome (ASPS) is a rare, genetically based sleep disorder characterized by an early evening onset of sleep, normal sleep duration, and spontaneous early awakening. The disorder leads to insomnia, excessive daytime sleepiness, and impairment of daily functioning and quality of life. The NHLBI supports basic research to elucidate the neural pathways through which the biological clock mechanism regulates sleep, clinical research to elucidate genetic risk factors, and applied research to determine the role of the biological clock in disturbed sleep and alertness of shift workers, school-age children, and drowsy drivers.

*Alpha-1 Antitrypsin Deficiency*

Alpha-1 antitrypsin (AAT) deficiency is an inherited deficiency of a circulating protein inhibitor that is manufactured primarily in the liver. The deficiency is associated with emphysema, presumably due to inadequate protection against enzymatic destruction of lung elastic fibers by neutrophil elastase. Fifteen percent of the AAT-deficient population also develops liver disease. The NHLBI funds clinical and basic research on AAT deficiency, including studies of the molecular mechanisms that impair AAT secretion, methods of gene therapy delivery, and methods to increase the availability of defective, but partially active, AAT. NHLBI-supported investigators are defining the abnormalities and degradation pathways of the AAT protein, characterizing the inflammation that leads to disease in various AAT deficiency states, and evaluating the possibility of treating the disease with drugs that would enhance the release of partially active mutant protein from liver cells. The objective of one genetic study of families is to identify other genes that may modify the nature and severity of the disease in different individuals. A gene therapy clinical trial is testing whether the AAT protein is produced in skeletal muscle cells after injection of a viral vector construct that includes the AAT gene. In addition to research that specifically focuses on AAT, the NHLBI supports related studies addressing lung transplantation; the general causation of emphysema; the function, synthesis, secretion, and interaction of the enzymes that are inhibited by AAT; animal models of other enzyme inhibitor deficiencies; gene regulation; cellular signaling, injury, and repair; and protein processing.

*Asbestosis*

Asbestosis, an occupational lung disease, is characterized by interstitial pneumonitis and fibrosis resulting from exposure to inhaled asbestos fibers. In response to the deposition of asbestos fibers, macrophages and lymphocytes accumulate, type II alveolar epithelial cells and smooth muscle cells proliferate, fibrosis appears in the adjacent walls of respiratory airways, and the alveolar septa thicken. Furthermore, asbestos fibers can be associated with cell transformation and proliferation related to lung cancers. NHLBI-supported researchers are investigating the molecular and cellular events that trigger cellular proliferation in and regulate remodeling of lung tissue, which results in fibrotic lesions and, perhaps, in malignant cell changes in response to asbestos. Recent advances in research related to asbestosis may help to explain why certain kinds of asbestos exposure (crocidolite) are associated with cancers. In experiments using epithelial cells, researchers recently showed that crocidolite asbestos depletes the intracellular antioxidant glutathione, causing increased production of an enzyme (gamma-glutamylcysteine synthetase) that increases levels of a cancer causing proto-oncogene. Another study showed that after exposure to asbestos, Type II lung epithelial cells activate extracellular signal-regulated kinases 1 and 2 (ERK1/2), which are stimuli for cellular proliferation.

*Bronchopulmonary Dysplasia*

Bronchopulmonary dysplasia (BPD) is a chronic lung disease characterized by disordered lung growth, changes in lung cell size and shape, and a reduction in the number of alveoli available for gas exchange. The NHLBI program in developmental lung biology supports basic and clinical research to increase understanding of BPD and identify treatment opportunities.

The Collaborative Program for Research in BPD provides a well-characterized primate model of BPD for a multi-disciplinary exploration of the molecular mechanisms involved in the etiology of BPD. One of the participating Centers of the NHLBI's SCOR Program in Pathobiology of Lung Development has identified nitric oxide (NO) as an important regulator of pulmonary circulation during development. The centers are conducting two clinical trials on the role of NO in preventing and treating chronic lung disease in premature infants. Together, the clinical studies are expected to yield definitive information about the utility and window of therapeutic opportunity for prevention of chronic lung disease with inhaled NO in very low-birth-weight premature infants. In addition, in FY 2004, the NHLBI entered into a funding collaboration with the National Institute of Child Health and Human Development to conduct a prospective, randomized study to test the individual factors of lower and conventional oxygen levels and two ventilatory strategies [conventional and nasal continuous positive airway pressure (nCPAP)] in very low-birth-weight, premature infants. The results of the study could exert broad influence on the clinical management of very low-birth-weight premature infants.

Several recent research advances may help scientists to discover new treatments for BPD. One study demonstrated a reduction of ventilatory injury with nCPAP in a preterm baboon model of BPD. In another study, a histological exam showed that baboon infants receiving nCPAP in place of conventional ventilation had more normal appearing, thinner, septated alveoli with associated capillary beds, although the absolute number of alveoli remained decreased compared to gestational age controls. Other recent work has shown that anti-bombesin antibody A211 promotes the growth of capillaries into developing alveolar septae, increasing the extent of alveolization in the 21-day baboon BPD model. Finally, analysis of the role of airway serpin (an antiproteinase) in balancing the activities of proteinases-antiproteinases during lung development reveals that proteinases are upregulated in BPD, whereas their local inhibitors are either downregulated or unchanged.

*Congenital Central Hypoventilation Syndrome*

Congenital central hypoventilation syndrome (CCHS) is a rare disorder characterized by normal breathing while awake but shallow breathing during sleep (hypopnea) that is not effective in moving fresh air into the lungs. In severe cases, breathing is also ineffective in affected individuals who are awake. The NHLBI supports a basic research program to elucidate the anatomical and physiological factors responsible for generating neural rhythm and translating it into breathing. Research is focused on understanding how breathing is regulated and identifying the conditions under which reflexive generation of respiratory rhythm is suppressed. Identification of the neuronal pathways producing respiratory rhythm and pattern is a prerequisite for a full understanding of a variety of respiratory sleep disorders such as CCHS. Genetic and pathology studies of CCHS patients are now leading to identification of candidate genes and of specific areas of the brain stem involved in autonomic regulation including respiration.

*Congenital Diaphragmatic Hernia*

Congenital diaphragmatic hernia (CDH) is a developmental disorder that occurs once in every 2,400 births. Often CDH occurs in isolated fashion, i.e., it is not associated with any other life-threatening anomalies or chromosomal aberrations.

Without surgical intervention neonates die soon after birth because their lung tissue, compressed by their herniated viscera, is inadequately developed. In infants with CDH, hypoplasia of the lung and its vascular bed leads to pulmonary hypertension or persistent fetal circulation syndrome. Recently, an investigator-initiated study funded by the NHLBI proposed to test the efficacy of an *in utero* surgical technique to correct lung hypoplasia in human fetuses with the most severe form of congenital hypoplasia. Enrollment was terminated on July 17, 2001, because of the unexpectedly high survival rate with standard care (postnatal surgery). The researchers determined that further recruitment would not result in significant differences between the standard care group and the *in utero* surgical group. Their findings of no difference in mortality, morbidity, and lung function between the two groups were published in the *New England Journal of Medicine* in FY 2004. The report was based on the results from 24 infants randomized before the study was terminated.

*Cystic Fibrosis*

Cystic fibrosis (CF) is a multi-system disease affecting a variety of epithelial tissues, characterized by defective transport of chloride and sodium across cell membranes. More than 25,000 Americans have CF, with an incidence of about 1 in 3,300 among Caucasians, making CF the nation's number one genetic cause of death for children and young adults. Defects in a single gene, the CF transmembrane conductance regulator (CFTR) gene, give rise to the disorder. The mutations in CFTR lead to abnormal secretions, recurrent infection and inflammation, bronchiectasis, and premature death. Lung disease is the major cause of morbidity and mortality in people with CF. Increasing evidence suggests that defects in the CTFR gene do not function alone in determining disease outcome. The severity of pulmonary disease can vary greatly among individuals, even in those with identical CTFR mutations. Evidence suggests that the variation is due to the interaction of the defects in the CTFR gene with other genes that can affect the final disease presentation.

The NHLBI supports a program of basic, clinical, and behavioral research in CF focused on the causes, pathophysiology, and treatment, specifically as it relates to the pulmonary manifestations. Four investigator-initiated program project grants in the area of gene therapy for CF were awarded in FY 2004. The grants are focused on developing improved vector delivery systems and overcoming barriers to gene therapy. In FY 2004, the NHLBI, in partnership with the National Institute of Diabetes and Digestive and Kidney Diseases, the Cystic Fibrosis Foundation (CFF), and several companies, sponsored an investigator-initiated multi-center clinical trial, Early Antipseudomonal Therapy in Cystic Fibrosis. It is the largest clinical trial involving young children with CF ever to be conducted in the United States and would be impractical without the Therapeutics Development Network (TDN) infrastructure, a unique resource for the CF research community, funded by the CFF and the NIH National Center for Research Resources (NCRR). The trial is seeking to determine the best treatment for initial *Pseudomonas* infection to delay or prevent chronic infections that lead to irreversible lung destruction and eventual death.

Two recent scientific advances have increased understanding of CF. For the first time, investigators have demonstrated that airway hydration plays a critical role in lung defense. Their results predict that rehydrating airway surfaces will be an effective form of therapy for CF and, perhaps, for chronic bronchitis and some forms of asthma as well. In their experiments,

overexpression of an epithelial sodium channel in mice caused accelerated sodium transport and initiated CF-like airway disease but did not affect chloride secretion, suggesting that an imbalance of sodium absorption and chloride secretion can produce CF-like disease. In another study, researchers focused on excess mucus production and secretion, a well-recognized feature of inflammatory respiratory diseases such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease. They discovered a key protein in the mucin secretory process (called MARCKS for myristolyated alanine-rich C kinase substrate) that regulates mucus secretion. When a peptide fragment from the protein, dubbed MANS, was created and tested in an animal model of disease, it sharply reduced or in some cases eliminated the excess mucin secretion, preventing the airways from clogging. The innovative findings lay a foundation for new therapies for a spectrum of respiratory diseases characterized by mucus hypersecretion.

*Idiopathic Pulmonary Fibrosis*

Idiopathic pulmonary fibrosis (IPF) is a rare, chronic, lung disease in which functioning normal lung tissue is replaced by nonfunctional connective (scar) tissue, which contains fibroblasts, myofibroblasts, and collagen. The causes of IPF are unknown, but the disease is commonly treated with corticosteroids, sometimes in combination with other immunosuppressant drugs, and less commonly with lung transplantation. Individuals with pulmonary fibrosis develop abnormal, excessive scarring that can cause progressive shortness of breath and cough. Therapy is rarely effective and the disease progresses, resulting in death over a relatively short time in most patients. NHLBI intramural researchers are conducting studies focusing on the natural history and pathogenesis of pulmonary fibrosis. Four intramural NHLBI observational clinical research protocols are enrolling individuals with pulmonary fibrosis. NHLBI-supported extramural researchers are investigating the molecular and cellular events that trigger the alveolar injury seen in early stage idiopathic pulmonary fibrosis that initiates progression to an irreversible, fibrotic end stage of the disease.

New research shows that fibroblasts involved in tissue fibrosis, which were previously believed to originate locally in lung tissue, originate in the bone marrow as progenitor fibroblasts. The finding, which indicates that local lung tissue injury can lead to a systemic response, has introduced new possibilities for treatment of IPF. Other recent scientific advances related to IPF include the discovery that a number of cytokines that cause fibroblasts to become activated and to proliferate are involved in the development of the disease. A great deal of research has focused on the transition of activated fibroblasts into myofibroblasts, which produce the growth factor TGF beta, a primary cause of dysregulated tissue repair. Recent research showed that the shift from activated fibroblast to myofibroblast is related to the presence of prostaglandins. Currently, TGF beta-secreting myofibroblasts are being targeted using immunosuppressant (interferon gamma) therapy. The treatment, which has been given to patients with newly diagnosed IPF, has produced modest improvement in younger patients with less severe disease. In another study, a murine model made deficient in leukotrienes was protected against fibrosis. A similar approach is being evaluated in IPF patients who are receiving an anti-leukotriene drug (Zileuton).

*Lymphangioleiomyomatosis*

Lymphangioleiomyomatosis (LAM) is a rare lung disease that affects women, usually during their reproductive years. Symptoms develop as a result of proliferation of atypical, nonmalignant, smooth muscle-like cells (LAM cells) in the lungs and in abdominal tumors (angiomyolipomas, lymphangioleiomyomas). Common symptoms include shortness of breath, cough, and sometimes coughing up blood. Patients often develop spontaneous pneumothorax or chylous pleural effusion (collapse of the lung or collection of milky looking fluid around the lung). The clinical course of LAM is quite variable but is usually slowly progressive, eventually resulting in death from respiratory failure. Although no treatment has been proven effective in halting or reversing LAM, lung transplantation is a valuable treatment for patients with end-stage lung disease. More than 30 percent of patients with tuberous sclerosis complex (TSC) develop lung lesions identical to those seen in LAM. The underlying genetic mechanisms leading to smooth muscle proliferation in LAM and TSC are controlled by abnormalities in the same genes, but TSC is inherited and LAM is a disease that occurs sporadically (does not appear to run in families).

As part of its intramural program, the Institute has established a research laboratory at the NIH Clinical Center to learn more about the cause and progression of LAM. Intramural researchers have been studying bone mineral density (BMD) in LAM patients, because LAM is frequently treated with anti-estrogen therapy (e.g., oophorectomy, progesterone), which can cause BMD loss. The researchers found that abnormal BMD, observed in roughly 70 percent of patients, was correlated with the severity of lung disease and with age. Greater severity of lung disease and oophorectomy therapy were associated with loss of BMD. Similar rates of decline in BMD were found in progesterone-treated and untreated patients. However, bisphosphonate-treated patients had lower rates of decline in BMD than did untreated patients. Based on these observations, the investigators recommended systematic evaluation of BMD and early treatment with bisphosphonates for patients with LAM.

The NHLBI extramural program supports a national LAM Patient Registry, co-funded by the Office of Research on Women's Health and coordinated by the Cleveland Clinic Foundation. The LAM Registry began enrolling patients in the summer of 1998. Enrollment closed in September 2001 with 243 patients recruited. The NHLBI LAM Registry program is continuing to help manage the collection, processing, and distribution of LAM tissue for current LAM projects as well as a repository of LAM tissue for future research. A recent study of blood vessels cells in benign angiomyolipomas in LAM patients showed that the vascular cells in these tumors are neoplastic. In addition, scientists recently showed for the first time that estrogen stimulates the growth of LAM-like cells from angioleiomyomas (benign kidney tumors). This may help to explain why LAM is almost exclusively a disease of women.

*Narcolepsy*

Narcolepsy is a disabling sleep disorder affecting over 100,000 people in the United States. It causes excessive daytime sleepiness and rapid onset of deep (REM) sleep. Other symptoms include abnormalities of dreaming sleep, such as dream-like hallucinations and transient periods of physical weakness or paralysis (cataplexy). Low cerebrospinal fluid levels of hypocretin, a neurochemical messenger linking sleep with the regulation of muscle tone and alertness, are highly

specific to narcolepsy. The NHLBI supports basic and clinical research to identify brain abnormalities associated with narcolepsy that contribute to symptoms such as daytime sleepiness, sleep disturbance, and physical weakness.

*Persistent Pulmonary Hypertension of the Newborn*

Persistent pulmonary hypertension of the newborn (PPHN) affects approximately 1 in 1,250 live-born term infants. Due to inappropriate muscularization of fetal pulmonary vessels, the lung arteries of affected newborns fail to dilate after birth to allow for normal blood flow through the lung. Infants with PPHN are poorly oxygenated and require costly and prolonged medical care including intubation of the airway, inhalation of 100-percent oxygen, mechanical ventilation and, often, heart/lung bypass (extracorporeal membrane oxygenation). The NHLBI supports a spectrum of basic and clinical research grants concerned with achieving a mechanistic understanding of structural and functional defects of the pulmonary circulation in order to create new opportunities for correcting them. One of four NHLBI-funded Specialized Centers of Research on the Pathobiology of Lung Development is studying several aspects of the unique vascular response of the neonate to injurious stimuli to identify basic molecular mechanisms involved in the development of hypertensive pulmonary disorders such as PPHN.

Several recent advances have been made in research related to PPHN. Recent clinical studies point to a critical role for endogenous nitric oxide as a modulator of levels of vasoactive mediators whose net balance determines pulmonary vascular tone and reactivity. New basic research studies indicate that a subpopulation of circulating monocytes (a type of white blood cell) are recruited to the pulmonary vasculature and contribute significantly to the accumulation of Type I collagen in the hypertensive vessel wall. Evidence is accumulating that at least some of these circulating progenitor cells differentiate into cells expressing a myofibroblast-like phenotype. Researchers have suggested that at least some forms of PPHN may result from abnormal accumulation of circulating pluripotent cells within the vessel wall. Thus, rational treatment of the disease depends on a mechanistic understanding of the fibroblast proliferative response observed in PPHN and may need to include therapies to inhibit the recruitment of fibroblasts to the vessel wall. In another recent clinical study, NHLBI-funded researchers performed a rigorous analysis of the data related to maternal risk factors for PPHN. Their analysis failed to support previously reported risk factors, such as smoking, while identifying some previously unrecognized risk factors. For example, third-trimester exposure to selective serotonin reuptake inhibitors (SSI) was associated with a two-fold increase in risk for BPD, whereas a 50-percent reduction in risk was associated with third-trimester exposure to ibuprofen. A six-fold increase in risk for PPHN was associated with C-section.

*Primary Ciliary Dyskinesia*

Primary ciliary dyskinesia (PCD), also known as Kartegener's syndrome or immobile ciliary syndrome, is an inherited disease characterized by defects in the cilia lining the respiratory tract. Patients with PCD exhibit impaired ciliary function, reduced or absent mucous clearance, and susceptibility to chronic, recurrent respiratory infections, including sinusitis, bronchitis, pneumonia, and otitis media. The disease typically affects children ages 0 to 18 years, but the defect associated with it has a variable clinical effect on disease progression in adults as well. Many patients experience hearing loss and, in males, infertility is common. Another symptom,

situs inversus (having organs on the opposite side from usual), occurs in approximately 50 percent of PCD patients. Clinical progression of the disease is variable, with lung transplantation required in severe cases. For most patients, aggressive measures to enhance clearance of mucus, prevent respiratory infections, and treat bacterial superinfections are recommended. NHLBI-supported researchers are relating the molecular etiology of PCD to the ciliary phenotype. A large cohort of subjects with PCD is undergoing rigorous diagnostic evaluation and determination of the specific ultrastructural abnormalities in their cilia. The relationship of mutations in specific genes to ultrastructural defects and ciliary functions is being investigated.

In a recent study, researchers evaluated diagnostic and phenotypic features in a large cohort of PCD patients to identify the major clinical and biologic markers of disease and to enable the categorization and characterization of patients. A combination of a careful clinical history and an examination of ciliary structural analysis and measures of biologic markers such as nasal nitric oxide was found to be useful in the diagnosis of PCD. Two other recent studies suggest that ciliated cells may have unique functions besides sweeping the airways; specifically, ciliated cells also appear to be equipped with specialized tools for transport and signaling. A regulatory factor (Foxj1), initially identified for its role in ciliogenesis in epithelial cells, has been shown to modulate immune lymphocyte differentiation, immune tolerance, and inflammation. A similar role for the regulatory factor in the airway epithelium would have profound implications since airway inflammation is important in lung disease. Finally, recent NHLBI-supported studies found that cell-associated enzymes from patients with PCD and other chronic lung diseases enhance nucleotide metabolism on airway epithelial cell surfaces. Enhancement of nucleotide metabolism could represent a protective mechanism against the deleterious effects of excess amounts of the nucleotide ATP. However, enhanced nucleotide metabolism and clearance also may exaggerate chronic inflammation in patients with PCD.

*Primary Pulmonary Hypertension*

Primary pulmonary hypertension (PPH) is a rare condition characterized by structural changes in small pulmonary arteries that lead to increased resistance to blood flow in the lungs and to heart failure. Over the past 25 years, PPH has been transformed from a poorly described disease with a dire prognosis to a well-defined illness that is being studied in basic investigations and clinical trials. Treatment with agents such as prostacyclins has improved the prognosis and quality of life of PPH patients, and newer treatment options such as endothelin inhibitors have recently become available. Despite recent advances, treatment is expensive and can have troublesome side effects. An important current priority is accelerating the translation of basic research advances into better therapies. The NHLBI supports research to identify the causes of PPH initiation and progression. Currently, the development of PPH is thought to require a genetic susceptibility followed by one or several secondary trigger factors such as viral infection or drug exposure. Determinants of genetic susceptibility and interaction of genotype with promoting or modifying factors are areas of active research. Other work is exploring the pathways within lung cells that contribute to the symptoms and pathology of PPH and the mechanisms underlying the abnormal proliferation of vascular cells in patients with PPH.

The discovery that a mutation in bone morphogenetic protein receptor 2 (BMPR2) is a genetic cause of PPH has sparked research on pathogenesis and therapeutic targets. Rapid progress is

being made in the search for a trigger or modifying condition for clinical presentation of BMPR2 mutations. The NHLBI supports a PPH Family Registry that was begun in 1994 to discover the genetic mechanism(s) of the disease. The registry currently comprises 81 families and has collected 324 samples for study. Research is determining whether variations in certain candidate genes contribute to development of the disease in genetically susceptible individuals. Current data from the registry suggest that roughly 50 percent of familial and 26 percent of nonfamilial (sporadic) PPH patients have mutations in the coding region of the BMPR2 receptor gene. As only 20 percent of individuals with a BMPR2 mutation develop PPH, other genetic factors likely play a role. Recent findings from the registry identified several possible modifier genes, including serotonin transporter, nitric oxide synthase 3, and vasoactive intestinal peptide, that may be risk factors for development of clinical disease or may in part determine the age of disease onset. Elucidating the genetic basis of PPH will enhance the ability of clinicians to inform affected family members about their risk of developing the disease. The basic research already funded by the NHLBI has enabled development of new agents that are currently being evaluated in clinical trials. So it is reasonable to expect that further basic research advances will lead to better treatments for PPH and other forms of severe pulmonary arterial hypertension.

*Sarcoidosis*

Sarcoidosis is a disease involving organ systems throughout the body in which normal tissue is invaded by pockets of inflammatory cells called granuloma. Most sarcoidosis patients have granuloma in their lungs. The disease can occur in a mild form that disappears spontaneously or in a severe form that results in a life-long condition. Estimates of the number of Americans with sarcoidosis range from 13,000 to 134,000, and between 2,600 and 27,000 new cases appear each year. Up to 5 percent of individuals with pulmonary sarcoidosis die of causes directly related to the disease. The morbidity associated with sarcoidosis can be severe, resulting in significant loss of function and decrease in quality of life. The causes of sarcoidosis are presently unknown, but disease development is thought to involve both a genetic predisposition and the immune system. Although corticosteroids are the mainstay of current treatment, alternative therapies could be beneficial given the multiple side effects that can arise from the use of corticosteroids.
The NHLBI supports research on sarcoidosis in both its extramural and intramural programs. Researchers in the NHLBI's intramural laboratories conduct translational studies relating to the effectiveness of cyclic nucleotide phoshodiesterase inhibitors and statins as potential new therapies for sarcoidosis. The NHLBI also funds extramural research grants to investigate the causes of sarcoidosis and the role of immune cell responses in the disease. As part of its extramural program, the NHLBI also supports a multicenter study, the U.S. Sarcoidosis Genetic Analysis Consortium (SAGA), to perform linkage analysis of 360 African-American families with affected siblings, using a 300-microsatellite marker scan. The focus is on African Americans as they are more likely to report a family history of sarcoidosis, present at an earlier age, and have more severe disease.

The NHLBI multi-center ACCESS (A Case Control Etiologic Study of Sarcoidosis) study created a repository of DNA specimens collected from more than 700 sarcoidosis patients and paired controls. A public access database for the repository is being prepared. In FY 2004, a publication by ACCESS investigators reported on social predictors of disease severity at presentation. The results showed that lower income, the absence of private or Medicare health insurance, and other

barriers to care were associated with sarcoidosis severity at presentation, as were race, sex, and age. Blacks were more likely to have severe disease by objective measures, while women were more likely than males to report subjective measures of severity. Another publication in FY 2004 focused on a 2-year follow-up study of a subset of 215 ACCESS patients. The investigators reported that about 80 percent of subjects had improved or stable pulmonary function. Patients with erythema nodosum at presentation were more likely to have improvement in the chest radiograph at 2-year follow-up. Patients with a lower annual family income were more likely to worsen with respect to dyspnea and were more likely to have new organ involvement at 2-year follow-up. The investigators concluded that in this group of sarcoidosis patients the disease tended to improve or remain stable over 2 years in the majority of patients. In a third ACCESS study, researchers interviewed participants (cases and controls) regarding occupational and nonoccupational exposures. Analysis of these data show some positive associations between sarcoidosis and specific occupations, e.g., agricultural employment, exposures to insecticides at work, environments with mold/mildew, and microbial bioaerosols. However, a history of smoking cigarettes was less frequent among cases than the controls. The study did not identify a single, predominant cause of sarcoidosis.

**Blood Diseases and Resources Programs**

*Acquired Aplastic Anemia*

Acquired aplastic anemia is an unusual hematologic disease in which the bone marrow fails to produce red cells, white cells, and platelets resulting in severe anemia, low white blood cell counts, and low platelet counts. The NHLBI Division of Intramural Research conducts clinical and laboratory research on bone marrow failure syndromes, including aplastic anemia. Intramural researchers have conducted multiple laboratory experiments directed at the pathophysiology of aplastic anemia as well as clinical programs dedicated to the treatment of the disease by immunosuppression and stem cell transplantation. Recently, the intramural program completed two clinical trials testing treatments for mild and severe aplastic anemia and a study of molecular clonotyping of the T-cell response in patients with aplastic anemia. They also described mutations in the telomere repair complex genes in patients with apparent acquired aplastic anemia.

*Cooley's Anemia*

Cooley's anemia (also called beta-thalassemia, thalassemia major, or Mediterranean anemia) is a genetic blood disease that results in inadequate production of hemoglobin. Individuals affected with Cooley's anemia require frequent and lifelong blood transfusions to sustain life. Because the body has no natural means to eliminate iron, the iron contained in transfused red blood cells builds up over many years and eventually becomes toxic to tissues and organ systems. Many children with Cooley's anemia have acquired other diseases such as hepatitis through years of transfusion exposure. Extramural research efforts of the NHLBI include identifying mutations in the globin gene cluster that lead to Cooley's anemia, determining the mechanism by which naturally occurring mutations significantly increase levels of fetal hemoglobin (Hb F) in adult red blood cells, developing therapeutic applications related to the naturally occurring mutations, studying iron chelation, identifying clinically useful therapies and drugs for the disorder, and developing gene therapy strategies to reduce morbidity and mortality associated with Cooley's anemia.

Currently, researchers are exploring new methods of transfusion therapies. For example, less toxic methods of stem cell transplantation are being developed that may be useful for patients with thalassemia. New iron chelators are also being evaluated. The Cooley's Anemia Clinical Research Network has designed protocols that will provide clinically useful information in the areas of hepatitis and new chelator development as well as insight into the potential utility of hemoglobin F induction. The network is completing a study measuring bone mass that shows that a significant decrease in bone mass occurs in all forms of thalassemia. The decrease in bone mass becomes progressively greater with age and is affected by weight and growth hormone deficiency.

Several new therapies are being developed to treat Cooley's anemia. Compounds that increase hemoglobin F levels have been described. The compounds include not only hydroxyurea, which is routinely used to treat sickle cell disease, but also a number of butyrate-based compounds as well as 5-azacytidine and decitabine. A new study is under way to compare the effects of deferoxamine (DFO) with deferiprone (L1) in reducing iron overload. As with other severe anemias associated with hemolysis, patients with thalassemia major suffer from pulmonary arterial hypertension (PAH) as demonstrated by increased tricuspid valve regurgitation velocities and by high pulmonary artery pressures. Patients with PAH are at higher risk of death resulting from acute pulmonary episodes including thromboembolisms. A new study is under way to test the efficacy of sildenafil in reducing PAH in thalassemia and sickle cell patients and thereby reducing morbidity and mortality.

*Creutzfeldt-Jakob Disease*

Creutzfeldt-Jakob disease (CJD) is a slow degenerative disease of the central nervous system that is characterized by motor dysfunction, progressive dementia, and vacuolar degeneration of the brain. The disease is rare but invariably fatal and is associated with a transmissible agent. A protease-resistant protein or prion is the hallmark of all transmissible spongiform encephalopathies (TSE) including CJD. Therefore, the term prion diseases is applied to this group of neurodegenerative illnesses, which includes bovine spongiform encephalopathy (BSE) or Amad cow disease,@ scrapie in sheep, and chronic wasting disease in deer and elk. Prion diseases may cross the species barrier, the most notable example being the recent cases of variant CJD (vCJD) in humans caused by consumption of beef contaminated with BSE. Classical CJD occurs worldwide at a rate of one to two cases per million per year.

The lack of a rapid, sensitive, and specific test for TSE infectivity has slowed progress in the study and control of CJD and other prion diseases. Development of assay systems that could be used for blood/tissue donor screening and for detecting disease in the preclinical stage is a high priority. The assays could also be useful in testing for TSE in animals, especially in domestic animals used for human consumption. A major goal of several NHLBI programs is to develop tests that detect TSE in asymptomatic individuals that would be suitable for screening the U.S. blood supply. The NHLBI and the National Institute of Neurological Disorders and Stroke are jointly supporting an extramural contract program to develop tests to detect TSE diseases. TSE agents are at such low concentrations in the blood of laboratory animals that they cannot be detected using current methods. Investigators are developing procedures to concentrate the agents so that they can be

detected with current assays. The NHLBI also supports a grant focused on developing a test to detect low levels of abnormal prion proteins based on fluorescence of the abnormal proteins.

*Fanconi Anemia*

Fanconi anemia (FA) is an autosomal-recessive bone marrow failure syndrome characterized by a decrease in blood cells and platelets, developmental defects, and cancer susceptibility. Many FA patients can be identified at birth because of congenital anomalies, although approximately 25 percent do not have birth defects. FA is a clinically heterogeneous disorder; it can currently be divided into at least eight complementation groups designated A through G. The NHLBI supports studies designed to identify and clone the remaining FA genes. An ongoing NHLBI program project has taken a multidisciplinary approach to identify causes of FA at the molecular and cellular level. The scientific areas represented in the program include molecular hematology, molecular genetics, mouse genetics, gene therapy, stem cell biology, and DNA repair. Continued efforts to develop protocols for the efficient identification and targeting of hematopoietic stem cells, to obtain information on how *ex vivo* manipulation of stem cells alters their biologic properties, and to improve vectors are expected to make significant contributions to understanding the pathophysiology of FA and enhancing the potential for a cure. Cord blood banking is another area of great potential benefit for hemoglobinopathy families. The NHLBI is currently supporting an investigator-initiated cooperative agreement to conduct sibling donor cord blood banking and transplantation.

Thus far, eight distinct forms of FA have been reported, suggesting that at least eight genes are involved in its manifestations. Two FA genes, FA-C and FA-A, account for an estimated 75 percent of all FA patients. More recent studies have identified a new FA complementation group (FA-L). FA-L is thought to play a critical action in gene repair. A number of studies are currently in progress to determine the causes of developmental abnormalities in FA. Ongoing studies are expected to continue to provide insight into the potential function of the abnormal proteins of Fanconi syndromes. The cellular localization of a functional protein complex that plays a role in DNA repair and prevention of mutagenesis have been exciting developments over the past few years. The interaction of the FA proteins with a breast cancer susceptibility gene (BRCA1) in a common pathway is another area of intense study. Rejection after allogeneic bone marrow transplantation for FA is another important research area since rejection remains a complication with a high risk of mortality. Recently, treatment with antilymphocyte globulin has shown promise for preventing rejection. In the study, rejection of a second allogeneic graft in a child with FA was reversed by antilymphocyte globulin and donor lymphocyte infusion.

*Hemophilia*

Hemophilia is a hereditary bleeding disorder caused by a deficiency in either blood coagulation factor VIII or factor IX. The approximately 20,000 individuals in the United States with hemophilia are dependent on lifelong treatment to control periodic bleeding episodes. The NHLBI supports a broad spectrum of activities on blood coagulation and its disorders. Hemophilia research includes viral and nonviral approaches for gene therapy, mechanisms of antibody inhibitor formation, modification of factors for improved therapeutics, safety of plasma-derived products, and blood product-associated infections. In addition, basic genetic, molecular biology, and protein biochemistry studies of factors VIII and IX are supported to improve understanding of

their mechanism and regulation. Two NHLBI-funded Program Project Grants support studies of gene-based therapies for hemophilia A and B and of other new therapies with a focus on treatment of patients who develop antibodies that neutralize replacement factor VIII.

Recently, NHLBI-supported investigators demonstrated the potential for using platelets as a source for the delivery and release of factor VIII protein at a site of injury. Scientists produced factor VIII-containing platelets from megakaryocytes expressing the factor VIII gene. Expression of factor VIII in platelets resulted in partial correction of the hemophilia A phenotype in mice. Investigators also are pursuing bioengineering strategies for improved secretion of factor VIII, which could enhance gene therapy approaches and increase production of recombinant factor VIII. A modified form of factor VIII was shown to be expressed 15- to 25-fold more efficiently than unmodified factor VIII. Other new research is exploring the use of activated factor VII in a gene transfer approach for treatment of hemophilia patients. Long-term expression and phenotypic correction of hemophilia B in mice was observed following adeno-associated virus (AAV) vector delivery of modified factor VIIa.

*Hereditary Hemorrhagic Telangiectasia*

Hereditary hemorrhagic telangiectasia (HHT) (or Osler-Weber-Rendu disease) is a bleeding disorder caused by weakness of the vascular support structure. Its most common manifestations are red spots on the lips and bleeding from mucosal membranes such as in the nose. In an advanced stage, arterio-venous malformations often develop in the lung, brain, gut, and liver. Two gene defects have been identified in patients with HHT. One is in a gene associated with the protein endoglin and the other is in a gene related to activin receptor-like kinase. A correlation may exist between the gene defect and organ susceptibility to the disease. The NHLBI supports a broad spectrum of research in hemostasis and thrombosis that is focused in part on understanding the biology of platelet activation, the mechanism of clotting, and the interaction of blood with the vascular surface.

*Lymphedema*

The lymphatic system regulates the flow of fluid that surrounds cells. Lymphedema occurs when the lymphatic system becomes unbalanced. The two major types of lymphedema are primary (congenital) lymphedema and secondary lymphedema (caused by tissue injury, scarring, cancer, lymph node removal, or infection). The NHLBI is interested in finding the developmental, molecular, and cellular causes of lymphedema as well as designing better therapies for both primary and secondary lymphedemas. Relevant NHLBI-funded projects include: Regulation of Angiogenesis by SLP-76 Signaling, Genes for Vascular Morphogenesis: a Genetic Approach, Influences of Lymph Flow on the Lymphatic Pump, Physiological Basis of Vascular Contractility, Lymph vs. Blood Angiogenesis: Functional Differences, Prox1 in Mammalian Lymphangiogenesis, FOXC2 in Hereditary Lymphedema, Control of the Lymphatic Endothelial Differentiation Program, and Molecular Characterization of Familial Lymphedema.

*Paroxysmal Nocturnal Hemoglobinuria (PHN)*

Paroxysmal nocturnal hemoglobinuria (PHN) is a disease of the bone marrow in which acquired, somatic mutations in the PIG-A gene lead to expansion of a clone of cells unable to express glycosylphosphatidylinositol-anchored proteins on their surface. The disorder results in intravascular hemolysis, proclivity to venous thromboses, and bone marrow failure. The NHLBI intramural program conducts clinical and laboratory studies in bone marrow failure syndromes, including PHN. Researchers in the intramural program of the NHLBI have used microarray technology to characterize the transcriptome in hematopoietic progenitor and stem cells of the PHN clone. They have also determined that clonal preleukemic diseases can arise in PNH patients from non-PIG-A-mutated clones. Currently, intramural researchers are participating in the planning of Alexion's clinical trial of eciluzamab, a monoclonal antibody of potential utility in blocking intravascular hemolysis.

*Sickle Cell Disease*

Sickle cell disease (SCD) is an inherited blood disorder that is most common among people whose ancestors come from Africa, the Middle East, the Mediterranean basin, and India. In the United States, approximately 50,000 individuals, primarily African Americans, have sickle cell disease (SS hemoglobin). One of every 650 African Americans (0.15 percent) is born with sickle cell disease, and about 8 percent are heterozygous for the sickle cell gene. Sickle cell disease occurs when an infant inherits the gene for sickle hemoglobin from both parents (Hb SS = sickle cell anemia) or the gene for sickle hemoglobin from one parent and the gene for another abnormal hemoglobin from the other parent (sickle cell disease types, e.g., Hb SC, Hb S-Beta thalassemia). In patients with the disease, the abnormal hemoglobin molecules tend to damage the red cells, causing them to stick to blood vessel walls. This leads to the acute painful episodes that are the hallmark of the disease. It also leads to chronic damage to the brain, heart, lungs, kidneys, spleen, and liver. The median age at death for patients with sickle cell disease is 42 years for men and 48 years for women. Pulmonary complications account for a large proportion of deaths among adults with sickle cell anemia. According to the Cooperative Study of Sickle Cell Disease (CSSCD), in a prospective multicenter study of 3,764 patients, over 20 percent of adults had fatal pulmonary complications of sickle cell anemia. Acute and chronic pulmonary complications of sickle cell anemia, such as pulmonary hypertension, pulmonary fibrosis, and asthma are also common.

Over the last 5 years the NHLBI, the NIH Clinical Center, and the National Institute of Diabetes and Digestive and Kidney Diseases have created a unified consortium of intramural investigators that is one of the largest sickle cell disease translational research programs in the country. The consortium has enrolled over 400 patients with sickle cell anemia and over 100 control subjects (48 African American) into six IRB-approved protocols. The largest of the protocols is addressing the prevalence, etiology, and treatment of secondary pulmonary hypertension, a leading cause of adult mortality in patients with sickle cell disease. The clinical program is complemented by a basic science program focusing on pathophysiology and experimental therapeutics. Current research in the NHLBI intramural program is determining the role of nitric oxide in the pathogenesis and treatment of sickle cell disease, characterizing the emerging syndrome of hemolytic anemia-associated secondary pulmonary hypertension, and identifying new therapeutic targets. In the last 2 years, three potential new therapeutic approaches (statin therapy, NO gas

inhalation, and sildenafil/L-arginine therapy) have been identified and are being addressed in ongoing clinical trials.

The current NHLBI sickle cell disease extramural research portfolio includes research on the following topic areas: (a) development of methods for gene addition to the hematopoietic stem cell, (b) characterization of interactions between sickled cells and the vascular endothelium, (c) improved understanding of hemoglobin gene switching to allow increased production of fetal hemoglobin, (d) a phase III clinical trial (STOP II) to evaluate the use of blood transfusion to prevent strokes in children with abnormal blood velocities, (e) a phase III clinical trial (BABY HUG) of hydroxyurea (HU) to determine if HU can prevent the onset of chronic end-organ damage in very young children with SCD, (f) production of an anti-B19 parvovirus vaccine for clinical trials in healthy volunteers and pediatric SCD patients, and (g) an epidemiologic study of adult patients who participated in the Multicenter Study of Hydroxyurea (MSH) Trial.

In FY 2004, NHLBI-funded investigators published papers on pulmonary hypertension in patients with SCD, blood mononuclear cell gene expression, biological activity of nitric oxide, and the role of inhaled nebulized nitrite. One highlight of research published in the past year was a report on the high prevalence of pulmonary hypertension and risk of death in adult patients with SCD. Despite having lower pulmonary-artery pressures and higher cardiac outputs than patients with primary pulmonary hypertension, patients with SCD and pulmonary hypertension had a significantly higher mortality rate than patients with SCD who did not have pulmonary hypertension. Researchers found that a tricuspid regurgitant jet velocity of at least 2.5 meters per second, as compared with a velocity of less than 2.5 meters per second, was strongly associated with an increased risk of death even after adjusting for other possible risk factors. The findings suggest that noninvasive measurement of tricuspid regurgitant jet velocity by echocardiography can be used to identify patients at high risk for death. The results support the use of Doppler echocardiographic screening in all adults with sickle cell disease to identify those patients at high risk who may benefit from intervention. Therapeutic trials of oxygen, warfarin, transfusion, and pulmonary vasodilator and remodeling medications are required to evaluate their potential to decrease the substantial morbidity and mortality associated with pulmonary hypertension.

Another important study published in 2004 evaluated the effects of the NHLBI-sponsored Stroke Prevention Trial in Sickle Cell Anemia (STOP). Although STOP demonstrated the efficacy of blood transfusions for primary stroke prevention in high-risk children SCD, the effect of this trial on public health had not been studied. Investigators in California recently sought to determine whether stroke rates in California among children with SCD have declined since the STOP findings were released in 1998. Using a state-wide hospital discharge database, they identified all first admissions for stroke in children with SCD from 1991 through 2000. The investigators observed declining stroke rates in children with SCD, coincident with the publication of the STOP study. Further studies are needed to demonstrate the causality of the association and to explore in greater detail both the implementation and benefit of transfusion therapy. The public health implications of this issue are great because of the substantial costs of blood transfusion therapy and the substantial potential benefit.

*Systemic Lupus Erythematosus (SLE)*

Systemic lupus erythematosus (SLE) is an autoimmune disorder in which the body produces antibodies that harm its own cells and tissues. Typical symptoms include fatigue, arthritis, fever, skin rashes, and kidney problems. SLE affects more women than men. The risk of coronary heart disease in women with SLE is up to 50 times higher than in the general population. SLE patients have a higher incidence of blood clot formation (thrombosis) and spontaneous loss of pregnancy. Although its cause remains unknown and no cure is currently available, SLE symptoms can be controlled with appropriate treatment so that most patients can lead an active life. The NHLBI supports two major areas of research relevant to SLE. First, the NHLBI funds research on components of the blood that regulate bleeding and blood clotting (hemostasis and thrombosis) to understand the biology of platelet molecule function, mechanisms of blood clotting, and the interaction of blood components with blood vessel (vascular) surfaces. Second, the NHLBI funds research on cardiovascular complications and risk factors that may help explain the elevated incidence of premature cardiovascular disease in women with SLE. Such factors include the presence of antibodies to phospholipid-binding proteins, which are present in 50 percent of SLE patients versus only 1–5 percent of healthy individuals. In addition, research is being conducted to study the role of inflammation and oxidative stress in SLE. One group of NHLBI-funded investigators recently completed studies to identify atherosclerotic risk factors in a young population of SLE patients. Another group of researchers conducted a cross-sectional study to measure atherosclerosis in women with SLE who did not have cardiovascular disease. The study found that risk factors associated with vascular stiffness seem to include some SLE-specific variables that are likely to be related to immune regulation.

*Thrombotic Thrombocytopenic Purpura*

Thrombotic thrombocytopenic purpura (TTP) is a potentially fatal disease characterized by low blood platelet levels and widespread platelet thrombi in arterioles and capillaries. The disease has a sudden onset and individuals with TTP often exhibit hemolysis, high fever, and neurological abnormalities. Management of patients with TTP is difficult due to the lack of specific diagnostic criteria and rapid progression of disease. The standard therapy for TTP is plasma exchange. Relapse after the acute phase is common. The clinical course differs significantly for patients with idiopathic TTP compared to patients with TTP provoked by predisposing conditions. A congenital or acquired deficiency of a plasma metalloprotease, ADAMTS 13, that cleaves large polymers of von Willebrand factor (vWF) has been linked to the disease. Despite advances in basic research on TTP, rapid and reliable assays for ADAMTS 13 for clinical use are lacking, treatment options are limited, and mortality remains high. The NHLBI supports a broad spectrum of research in hemostasis and thrombosis that includes research on platelet biology, blood coagulation, and the interaction of blood with the vascular surface. Recently, grants specifically targeted to TTP have been awarded. In addition, efforts are under way to develop rapid and specific assays for ADAMTS 13 and to produce recombinant ADAMTS 13 on a large scale.

NHLBI-supported grantees have made fundamental contributions toward the discovery of ADAMTS 13 and the understanding of TTP. The observation that an antibody to ADAMTS 13 may cause TTP was a major breakthrough in the field. Researchers now know that a deficiency of

the protease causes both congenital and acquired TTP (antibody induced). A recent study demonstrated variable performances of available assays that measure ADAMTS 13 but provided an optimistic view about the reliability of currently available methods for measuring the protease level. Recently, a new enzyme immunoassay (EIA) for measuring the activity of ADAMTS 13 in plasma samples of patients has been described. The EIA could distinguish patients from normal individuals or carriers of one copy of mutant ADAMTS 13 allele. The EIA method is simple and could be adapted for general use in clinics. Overall, new assays correlate well with diagnostic categories, the response to plasma exchange, and the likelihood of relapse. A recent study showed that inflammatory cytokines may regulate the processing of vWF by ADAMTS 13. The finding may lead to new therapeutic interventions for patients with TTP and other inflammation-associated thrombotic disorders.

**Rare Diseases Research Initiatives**

**Ongoing Initiatives**

- Animal Models of Antigen-Specific Tolerance for Heart and Lung Transplantation
- Beryllium-induced Diseases
- Biology of Iron Overload and New Approaches to Therapy
- Blood and Marrow Transplant Clinical Research Network
- Cell-based Therapies for Heart, Lung, Blood, and Sleep Disorders and Diseases
- Cellular and Molecular Mechanisms of Primary Pulmonary Hypertension
- Chemical and Genetic Screens for New Inducers of Fetal Hemoglobin Genes for Treatment of Sickle Cell Disease and Cooley's Anemia
- Chemical Genomics and Molecular Libraries for Sickle Cell Disease
- Chronic Fatigue Syndrome: Pathophysiology and Treatment
- Comprehensive Sickle Cell Centers
- Coordination of Vascularization and Lung Development
- Cord Blood Transplantation Study
- Developmental Processes in Differential Expression of Globin Genes
- Development of an Assay for Crutzfeldt-Jakob Disease
- Diamond-Blackfan Anemia and Other Congenital Bone Marrow Failure Syndromes: Underlying Molecular Mechanisms
- Functional Heterogeneity of the Peripheral, Pulmonary, and Lymphatic Vessels
- Genelink
- Genetic Aspects of Tuberculosis in the Lung
- Genetic Modifiers of Single Gene Defect Diseases

- Granulomatous Lung Inflammation in Sarcoidosis
- Hemophilia and Hereditary Bleeding Disorders: Improved Therapy
- Heritable Disorders of Connective Tissue
- Hutchinson-Gilford Progeria Syndrome: Exploratory/Developmental (R21) Grants
- Idiopathic Pulmonary Fibrosis Clinical Research Network
- International Cooperative Biodiversity Groups (ICBG)
- Marfan Syndrome: National Registry
- Mechanisms of Fetal Hemoglobin Gene Silencing for Treatment of Sickle Cell Disease and Cooley's Anemia
- Mesenchymal Stem Cell Biology
- Molecular Mechanisms of Mucous Cell Metaplasia and Excess Mucous Secretion in Human Airway Diseases
- Multicenter Study of Hydroxyurea in Sickle Cell Disease: Patient Follow-Up Extension I
- Myelodysplastic Syndromes (MDS): Pathogenesis and Disease Progression
- Myeloproliferative Disorders (MPD): Pathogenesis and Disease Progression
- NHLBI Clinical Proteomics Programs
- NHLBI Lung Tissue Resource
- Novel Approaches to Enhance Animal Stem Cell Research
- Oxygen Sensing During Intermittent Hypoxia
- Pathogenesis and Treatment of Lymphedema and Lymphatic Diseases
- Pathogenesis of SARS Lung Disease: *In vitro* Studies and Animal Models
- Pediatric Heart Disease Clinical Research Network
- Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)
- Pediatric Mechanical Circulatory Support
- Plasticity of Human Stem Cells in the Nervous System
- Programs for Genomic Applications (PGAs) for Heart, Lung, and Blood Research
- Programs of Excellence in Gene Therapy (PEGT) for Heart, Lung, and Blood Diseases
- Pulmonary Complications of Sickle Cell Disease
- Pulmonary Fibrosis: Molecular Targets and Interventions
- Rare Diseases: Exploratory and Developmental Research Grants
- Sickle Cell Disease Clinical Research Network
- Sildenafil for Sickle Cell-Associated Pulmonary Hypertension: Phase II/III Clinical Trial

- Somatic Cell Therapy Processing Facilities
- Specialized Centers of Clinically Oriented Research (SCCOR) in Pediatric Cardiovascular Disease
- Specialized Centers of Clinically Oriented Research (SCCOR) in Pulmonary Vascular Disease
- Specialized Centers of Research (SCOR) in Hematopoietic Stem Cell Biology
- Specialized Centers of Research (SCOR) in (a) Neurobiology of Sleep and Sleep Apnea and (b) Airway Biology and Pathogenesis of Cystic Fibrosis
- Specialized Centers of Research (SCOR) in Pathobiology of Fibrous Lung Disease
- Stem Cell Plasticity in Hematopoietic and Non-hematopoietic Tissue
- Thalassemia (Cooley's Anemia) Clinical Research Network
- Transactivation of Fetal Hemoglobin Genes for Treatment of Sickle Cell Disease and Cooley's Anemia
- Transfusion Medicine/Hemostasis Clinical Research Network
- Treatment of HIV and Associated Complications in Hemophiliacs
- Tuberculosis Curriculum Coordinating Center
- Vascular and Hematopoietic Development and Disease

**Initiatives Started in 2004**

*Diamond-Blackfan Anemia and Other Congenital Bone Marrow Failure Syndromes: Underlying Molecular Mechanisms*

A new NHLBI-initiated Request for Applications (RFA) promotes research on the genetics and biochemical mechanisms of Diamond-Blackfan anemia and other rare inherited bone marrow failure syndromes. An understanding of the molecular pathways disrupted in the syndromes will facilitate development of targeted therapies and stimulate research on the molecular mechanisms underlying defective hematopoiesis, congenital anomalies, and cancer development in marrow failure diseases. Other diseases that are related to this initiative include dyskeratosis congenita, Pearson syndrome, severe congenital neutropenia (Kostmann syndrome), Shwachman-Diamond syndrome, and congenital amegakaryocytic thrombocytopenia.

*Granulomatous Lung Inflammation in Sarcoidosis*

A new NHLBI-initiated RFA supports research on the immunopathogenic mechanisms that lead to a nontuberculous granulomatous inflammation in the lungs of individuals with sarcoidosis. Research focuses on investigating the etiology of sarcoidosis, determining its susceptibility factors, and identifying the components of the innate and/or adaptive immune pathways that affect lung lymph nodes and tissue.

*NHLBI Lung Tissue Resource*

A new NHLBI-sponsored Broad Agency Announcement (BAA) establishes and supports a program for the standardized processing, storage, and distribution of lung tissues and associated clinical data. The resources will enable investigators to perform studies correlating molecular histopathology of the lung with pulmonary function and clinical status. Tissues will be procured and processed from 500 smoking and nonsmoking subjects with essentially normal lungs and from 2,000 subjects with pulmonary disease. The majority of those with disease will have COPD and will be extensively characterized with respect to airflow limitation and CT measures of emphysema. Lung specimens will also be obtained, when possible, from individuals with pulmonary fibrosis, sarcoidosis, asthma, primary pulmonary hypertension, and other serious chronic diseases that affect the lungs.

*Pathogenesis and Treatment of Lymphedema and Lymphatic Diseases*

A renewal of an NHLBI-initiated, trans-NIH Program Announcement (PA) investigates the pathogenesis of, and new treatments for, primary and secondary lymphedema. Lymphedema may be a congenital condition or may arise as a result of surgery, radiation, or the presence of a tumor in the area of the lymph nodes. Research will focus on the biology of the lymphatic system, the pathophysiologic mechanisms that cause lymphedema and lymphatic diseases, methods for quantitating and imaging lymph flow, therapeutic interventions, and mechanisms of action of complementary and alternative therapies. New knowledge in lymphatic disease research should improve early diagnosis of affected individuals, choice and timing of treatment, and genetic counseling.

*Pediatric Mechanical Circulatory Support*

A new BAA, initiated by the NHLBI, supports development of mechanical assist devices, including extracorporeal membrane oxygenation (ECMO) systems, left ventricular assist devices (LVADs), and other bioengineered systems for children with congenital and acquired cardiovascular disease. The program provides basic physiological and bioengineering data necessary for the design of effective pediatric assist and replacement devices while also supporting phase I studies to explore innovative strategies to meet the clinical needs of the pediatric patient population.

*Programs for Genomic Applications for Heart, Lung, and Blood Research*

A renewal of an NHLBI-initiated RFA links the genomic resources and tools of the Human Genome Project (HGP) to studies of major biological processes and systems involved in cardiovascular, pulmonary, hematologic, and sleep function and dysfunction through the establishment of 11 Programs for Genomic Applications (PGAs) for Heart, Lung, and Blood Research. The PGAs identify subsets of genes that are particularly relevant to the biology, diagnosis, management, treatment, and prevention of heart, lung, blood, and sleep-related disorders and prioritize the information for further focused study. The generation and interpretation of data from the PGAs enables a broad range of investigators to exploit the opportunities provided by the

HGP and related technologies. In addition, the PGAs support training and education programs for NHLBI-supported investigators in the use of genomic information and technologies. The 11 PGAs will continue to collaborate to develop common protocols, standard procedures, and nonredundant education and training efforts.

*Rare Diseases: Exploratory and Developmental Research Grant*

A new PA, initiated by the NHLBI and cosponsored by the Office of Rare Diseases, encourages research on understanding, treating, and preventing rare heart, lung, and blood diseases as well as sleep disorders. Rare diseases are often referred to as "orphan" diseases since there is a general lack of interest among industries to invest resources in diseases that in aggregate affect too few people to guarantee a reasonable return on investment. The availability of R21 exploratory and developmental awards is expected to allow investigators with new ideas to obtain research support without the need for large amounts of preliminary data, a requirement that can serve as a barrier to obtaining other types of awards.

**Initiatives Planned for the Future**

*Chronic Fatigue Syndrome: Pathophysiology and Treatment*

A renewal of a trans-NIH PA, sponsored in part by the NHLBI, will investigate the pathophysiology and treatment of chronic fatigue syndrome (CFS) in diverse groups and across the life span to improve the diagnosis, treatment, and quality of life of all persons with CFS. Research on new hypotheses, heterogeneous population groups, research gaps, and common mediators influencing the actions among and between various bodily systems will be encouraged. Topics of special interest to the NHLBI include: (1) the role of neuro-cardiovascular regulation in the loss of normal control of blood pressure, heart rate, and contractility in CFS patients and (2) factors and mechanisms involved in altered sleep state, circadian regulation, and other causes of impaired or ineffective sleep in CFS patients.

*Hemophilia and Hereditary Bleeding Disorders: Improved Therapy*

A new RFA, to be initiated by the NHLBI in FY 2005, will improve understanding of immune response and safety issues related to gene transfer or cell-based therapies for bleeding disorders. The objective of the research is to develop improved treatments and possibly cures for hemophilia, von Willebrand disease, and other hereditary bleeding disorders. The initiative is co-funded by the National Hemophilia Foundation, an organization promoting education, research, and advocacy on behalf of people with bleeding disorders.

*Idiopathic Lung Fibrosis Clinical Research Network*

In FY 2005, a new NHLBI-initiated RFA will establish a network of clinical centers to conduct multiple treatment trials on patients with established idiopathic pulmonary fibrosis (IPF), a disease of inflammation that results in scarring of the lungs and eventually interference with oxygen transport. Researchers believe that IPF may result from either an autoimmune disorder or from

infection, most likely by a virus. The network will consist of approximately 10 clinical centers and a data coordinating center. Each of the clinical centers is expected to enroll 40 to 50 patients per year for a 2-year interval of treatment and 2 years of follow-up. For patients who require an open lung biopsy for diagnosis, living lung tissue and blood will be stored for future studies on cellular genomic and immunopathogenic changes.

*Marfan Syndrome: National Registry of Patients*

A new RFA will be initiated by the NHLBI and cosponsored by the NIAMS in FY 2005 to establish a registry to collect and analyze clinical data and samples (e.g., blood and tissue) from patients with Marfan syndrome and to improve understanding of cardiovascular complications and therapies for the disorder. Ultimately, the registry will provide an essential resource to improve clinical care for patients.

*Muscular Dystrophy: Pathogenesis and Therapies*

In FY 2005 a new PA, sponsored in part by the NHLBI, will investigate the pathogenesis of muscular dystrophies (MD) and develop therapies for them. Premature death due to MD is often caused by cardiac or respiratory failure. Though recent research has increased knowledge about genetic defects associated with many forms of MD, a corresponding improvement in the treatment of MD has not been achieved. Important research priorities include studies of gene and stem cell therapies, pharmacological approaches to treatment, and clarification of the role of inflammatory mechanisms.

*Myeloproliferative Disorders: Pathogenesis and Disease Progression*

A new RFA, to be initiated by the NHLBI in FY 2005 and cosponsored by the NCI, will support research on the genetic, biochemical, and molecular pathways that operate in the emergence and progression of myeloproliferative disorders (MPD), a group of conditions characterized by excessive proliferation and production of one or more of the bone marrow (myeloid) cells. Research will focus on analyzing genes that are expressed in MPD and identifying gene products that are associated with or responsible for disease development and progression to a malignant and fatal outcome. Such discoveries will be critical to the development of new therapies for MPD patients who are not suitable candidates for hematopoietic stem cell transplantation, the only curative therapy currently available.

*NHLBI Clinical Proteomics Programs*

In FY 2005, the NHLBI will initiate a new RFA to validate on a systematic, comprehensive, large-scale basis existing and new candidate protein markers that are appropriate for routine use in the diagnosis and management of heart, lung, blood, or sleep diseases and disorders. The initiative establishes an infrastructure for research teams to validate protein panels that may be used to predict disease susceptibility or to assist in differential diagnosis, disease staging, selection of individualized therapies, and monitoring of treatment responses. The validation process will use disease-associated biological samples and clinical data from ongoing and completed NHLBI clinical trials and epidemiologic studies. In addition, the programs will provide education and

skills development to ensure that scientists have the competencies and expertise needed to address the multifaceted challenges of clinical proteomics.

*Novel Approaches to Enhance Animal Stem Cell Research*

In FY 2005, the NHLBI will collaborate with several Institutes to fund the renewal of a PA to support the identification, isolation, and characterization of totipotent and multipotent stem cells from biomedical animal research models. The research will generate reagents and develop techniques to characterize and separate stem cells from other cell types. The PA stresses innovative approaches to the problems of making multipotent stem cells available from a variety of non-human sources, creating reagents that will identify multipotent stem cells across species and allow for their separation from differentiated cell types.

*Pathogenesis of SARS Lung Disease: In Vitro Studies and Animal Models*

A new PA will be initiated by the NHLBI in FY 2005 to advance understanding of the pathogenesis of severe acute respiratory syndrome (SARS) in the lung using *in vitro* techniques, existing animal models of related coronavirus infections, non-human primate models of SARS, and new rodent models. Research topics of interest include *in vitro* research on the role of collectins and other extracellular host factors; studies of endothelial and epithelial permeability; and investigations of the effects of SARS on fluid movements, growth, and differentiation of human lung cells (e.g., alveolar epithelial cells, fibroblasts). The primary focus is on human SARS coronaviruses, but research using engineered and related animal coronaviruses and animal cells pertinent to the pathogenesis of SARS may also be conducted.

*Programs of Excellence in Gene Therapy for Heart, Lung, and Blood Diseases*

A renewal in FY 2005 of an NHLBI-initiated RFA will promote the rapid translation of basic, preclinical studies of gene therapy for cardiovascular, pulmonary, and hematologic diseases into pilot studies in humans. During their second 5-year operating period, the NHLBI Programs of Excellence in Gene Therapy (PEGT) will: (1) conduct preclinical projects to facilitate the translation of gene therapy into clinical studies; (2) conduct clinical phase I/II studies to test the safety and efficacy of gene therapy procedures; (3) operate six national cores to provide no-cost resources and services to NHLBI-supported investigators conducting gene therapy research; and (4) train MD, MD/PhD, and PhD scientists in conducting gene therapy clinical trials.

*Pulmonary Complications of Sickle Cell Disease*

In FY 2005, a new RFA will be initiated by the NHLBI to conduct basic and clinical research on pulmonary complications of sickle cell disease (SCD). Acute sickle cell chest syndrome, the second most common acute clinical complication of SCD, is characterized by infiltrates in the lungs and sometimes by fever, pneumonia, and thromboembolism of peripheral blood clots and/or fat emboli. The less common chronic form of sickle cell lung disease is characterized by pulmonary perfusion and diffusion defects, pulmonary hypertension, changes in the vessel walls such as intramural and perivascular connective tissue deposition, hyperplasia/hypertrophy of

smooth muscle cells, and in some cases by intramural thrombosis. Further elucidation of the acute and chronic lung syndromes is required in order to develop more adequate therapies.

*Thalassemia Clinical Research Network*

In FY 2005, the NHLBI will renew an RFA to continue operation of a cooperative network of five clinical centers and a data coordinating center conducting clinical trials to evaluate existing and future therapies for the treatment of thalassemia major (Cooley's anemia). The network enhances progress in moving effective therapies, e.g., fetal hemoglobin enhancing agents, gene therapy, or iron chelation, from the laboratory to the bedside through rapid and systematic collaborative testing in phase II and phase III clinical trials. A registry of thalassemia patients has also been developed and will be used to identify patients available for future trials.

*Sickle Cell Disease Clinical Research Network*

A new RFA, which will be initiated by the NHLBI in FY 2006, will establish a clinical research network to conduct multiple phase III randomized, controlled clinical trials to test the efficacy of new therapies to treat and prevent complications of sickle cell disease and, when appropriate, thalassemia. The interventions will be based on results from basic studies and phase I and phase II clinical trials conducted in programs such as the NHLBI Comprehensive Sickle Cell Centers Program. The network will comprise a data coordinating center and up to 15 clinical centers that will enroll 50 or more patients per center per year to participate in multiple trials using common protocols. In addition, the network is designed to create datasets that can be used to characterize patients and their clinical course, apply genomic and proteomic techniques for improved diagnostic and therapeutic approaches, expand clinical application of multimodal therapies, and examine patient-centered outcomes.

*Specialized Centers of Clinically Oriented Research in Pulmonary Vascular Disease*

In FY 2005, the NHLBI will initiate a new RFA to conduct multidisciplinary research on clinical questions related to the diagnosis, prevention, and treatment of pulmonary vascular disease. The program will address primary (idiopathic) and secondary pulmonary arterial hypertension, acute and chronic pulmonary thromboembolism, right ventricular dysfunction, and pulmonary vascular disorders of infants and children. Three to four centers will each carry out a minimum of three research projects directly related to a unifying theme. At least half of the projects in each center will be clinically oriented to assure that basic science findings are rapidly applied to clinical problems. Applicants may propose inclusion of a Clinical Research Skills Development Core to enhance the research skills of new clinical investigators.

**Rare Disease-related Program Activities**

*Alpha-1 Antitrypsin Deficiency*

The NHLBI sponsored an education strategy development workshop on chronic obstructive pulmonary disease on September 22–23, 2004, in Arlington, Virginia. The purpose of the meeting

was to develop priorities for improving awareness and knowledge of alpha-1 antitrypsin (AAT) deficiency and chronic obstructive pulmonary disease (COPD).

The NHLBI held a workshop titled "Critical Issues in COPD Research" on September 28–29, 2004 in Bethesda, Maryland. Recommendations were made regarding priorities for future scientific research on AAT deficiency-related lung disease and COPD.

*Arrhythmogenic Right Ventricular Dysplasia*

NHLBI-funded investigators have produced an informational brochure titled "Physicians Guide to Diagnosis of ARVD" and distributed it to the membership of the Heart Rhythm Society.

NHLBI-funded investigators recently produced a third volume of the newsletter "Lo Que Pasa, Newsletter of the Multidisciplinary Study of Right Ventricular Dysplasia."

*Bronchopulmonary Dysplasia*

The NHLBI sponsored the annual meeting of the Collaborative Program for Research in BPD, which was held on September 23–24, 2004, in Bethesda, Maryland.

*Congenital Central Hypoventilation Syndrome*

The NHLBI cosponsored the Eighth International Conference on Sleep and Breathing on October 13–16, 2004, in Newport, Rhode Island. Many of the discussions were directly related to the control of breathing in a variety of sleep disorders.

*Congenital Heart Disease*

Five Pediatric Circulatory Support contracts were awarded in 2004. The five contracts provide for development of a wide range of pediatric ventricular assist devices (VADs): a fully implantable, miniature centrifugal pump; infant- and child-sized pulsatile flow VADs; an innovative, small, extracorporeal membrane oxygenation device; a miniature axial flow VAD for the entire range of pediatric patient sizes; and infant- and child-sized axial flow pumps based on a more standard VAD design.

The Weinstein Cardiovascular Development Conference is an annual meeting partially supported by NHLBI. The 2004 meeting was held on May 13–16, 2004, in Noordwijkerhout, The Netherlands. The meeting included reports by NHLBI-supported investigators on congenital heart disease.

*Cooley's Anemia*

On April 17, 2004, the NHLBI convened a meeting titled "Future Directions in the Hemoglobinopathies" to bring together investigators active in research on hemoglobin disorders. Attendees discussed the feasibility of a future clinical research network for both SCD and beta-thalassemia. Currently, the NHLBI supports two separate networks—one for SCD and one for

thalassemia. However, several research topics now under study have therapeutic potential for both diseases. While both communities have some specific concerns, the overall concept was endorsed by the attendees.
The NHLBI held a meeting for investigators with projects funded through RFAs related to hemoglobin disorders on September 22–24, 2004.

On October 4, 2004, a number of investigators supported by the NHLBI and the NIDDK met to discuss progress in their research to improve the utility of MRI as a method for quantitative determinations of tissue iron, especially in liver, heart, and brain.

*DiGeorge Syndrome*

The Weinstein Cardiovascular Development Conference is an annual meeting partially supported by the NHLBI. The 2004 meeting was held on May 13–16, 2004, in Noordwijkerhout, The Netherlands. The meeting included reports by NHLBI-supported investigators on congenital heart disease.

*Fanconi Anemia*

The fifteenth annual FA Research Fund Scientific Symposium was held on October 16–19, 2003, in Houston, Texas, with 46 researchers presenting to over 150 participants from 11 countries. The meeting was sponsored by the Fanconi Anemia Research Foundation.

*Hemophilia*

A recombinant porcine Factor VIII is being developed by Octagen Corporation, with SBIR support, as a treatment option for hemophilia A patients who develop antibody inhibitors that neutralize the activity of currently available types of replacement Factor VIII.

*Long QT Syndrome*

Several NHLBI-funded scientists participated in developing new guidelines to identify and prevent acquired LQTS from occurring as an unwanted side effect of drug therapy.

*Lymphangioleiomyomatosis*

The NHLBI, the Office of Rare Diseases, and the LAM Foundation cosponsored the LAM Research Conference in Cincinnati, Ohio, March 26–28, 2004.

*Lymphedema*

The first ever Gordon Conference on lymphatic biology titled “Molecular Mechanisms in Lymphatic Function and Diseases,” sponsored in part by the NHLBI, was held March 7–12, 2004 in Ventura, California.

*Primary Pulmonary Hypertension*

The Pulmonary Hypertension Association (PHA) is collaborating with the NHLBI to support new Career Development Award (K08-K23) investigators who are conducting research projects on PPH.

The NHLBI provided support for the 2004 Grover Conference on "Genetic and Environmental Determinants of Pulmonary Endothelial Cell Function," which was held on September 9–12, 2004, in Sedalia, Colorado.

In collaboration with the NIH Office of Rare Diseases and the Centers for Disease Control and Prevention, the NHLBI provided funding for the "PHA International Pulmonary Hypertension Conference—Scientific Session" held on June 24–27 in Miami, Florida.

*Sarcoidosis*

The NHLBI is revising the Sarcoidosis Fact Sheet.

A representative from the NHLBI is chairperson of the recently established trans-NIH Sarcoidosis Working Group. The first meeting of the working group was held on November 1, 2004. The group is reviewing current NIH-funded research pertinent to sarcoidosis and discussing the possibility of future joint activities and initiatives.

*Sickle Cell Disease*

NHLBI investigators are participating in research related to a CRADA agreement between the NIH Clinical Center and INO Therapeutics. The CRADA supports a large screening trial to identify sickle cell patients with pulmonary hypertension and to treat them with inhaled NO gas. The results of the screening trial, which were published in the *New England Journal of Medicine*, revealed that 30 percent of sickle cell patients develop pulmonary hypertension, which is the leading cause of death in sickle cell patients.

The Comprehensive Sickle Cell Centers Steering Committee met for the fourth time on December 4, 2003, to present, discuss, and rank proposals for collaborative multicenter clinical research protocols. The Steering Committee selected 10 protocols for further development: oral arginine supplementation in children and adults; neurocognitive function in adults; a comprehensive patient registry, including epidemiology of headaches; priapism—prevalence and prevention; hydroxyurea plus magnesium for hemoglobin sickle cell disease; decitabine for fetal hemoglobin induction; pain treatment with methadone; interventional trial for headaches; interventional trial for priapism; and dexamethasone for acute chest syndrome.

On April 17, 2004, the NHLBI convened a meeting titled "Future Directions in the Hemoglobinopathies" to bring together investigators active in research on hemoglobin disorders. Attendees discussed the feasibility of a future clinical research network for both SCD and beta-thalassemia. Currently, the NHLBI supports two separate networks—one for SCD and one for thalassemia. However, several research topics now under study have therapeutic potential for both

diseases. While both communities have some specific concerns, the overall concept was endorsed by the attendees.

The NHLBI convened the "Sickle Cell Consultative Network Meeting" on September 21, 2004. Adult hematologists who care for patients with sickle cell disease met to discuss improving care by providing consultation services for patients who live in parts of the United States that are not served by a Comprehensive Sickle Cell Disease Center.

On September 22–24, 2004, 25 investigators with projects funded through any one of several NHLBI RFAs related to hemoglobin disorders met to discuss research progress.

The BABY HUG Steering Committee met on September 23, 2004, to discuss progress in the ongoing pilot phase of this clinical trial. The objective of the BABY HUG trial is to determine if hydroxyurea therapy is effective in the prevention of chronic end organ damage in young patients with sickle cell anemia. The clinical trial will involve the cooperation of pediatric clinical centers with a medical coordinating center that will supervise drug distribution, central laboratory functions, and data collection.

The MSH Patients' Follow-up Steering Committee Meeting met on September 24, 2004, to discuss follow-up of the MSH cohort for the next 5 years. A paper summarizing survival for the last 9 years has been published. Data from the cohort suggest that survival is improved if fetal hemoglobin levels are elevated by continuing hydroxyurea therapy.

On September 22, 2004, the STOP II Trial Steering Committee met to discuss the STOP II Trial protocol and subject recruitment. The objective of STOP II is to determine whether it is safe to stop transfusing children after 30 months for stroke prevention. The trial is now in its fourth year of funding and will be terminated early due to the accumulation of significant evidence against stopping transfusion at 30 months.

**Problem Areas Related to Rare Diseases**

*Advanced Sleep Phase Syndrome*

Because advanced sleep phase syndrome (ASPS) is a rare genetic disorder, it is difficult to recruit patients for clinical studies.

*Alpha-1 Antitrypsin Deficiency*

Research needs include better animal models of the disease, identification of biomarkers, identification of modifier genes, development of chemical chaperones that could specifically enhance the secretion of mutant alpha-1 antitrypsin protein, improvements in approaches to gene therapy, and new treatments to slow the progression of emphysema.

*Acquired Aplastic Anemia*

More research is needed to increase the response to immunosuppressive treatment, prevent relapse by improving immunosuppressive regimens, prevent the evolution of late clonal disease, describe fully the clonotypes among dominant T cells, and characterize the role of telomere repair complex genes in maintenance of normal hematopoiesis.

*Arrhythmogenic Right Ventricular Dysplasia*

The Multidisciplinary Study of Right Ventricular Dysplasia has had many challenges in recruiting patients/family members, primarily due to IRB delays at potential new centers and difficulty with screening families of interest due to new HIPPA requirements. Steps to streamline enrollment of family members have been put in place.

*Bronchopulmonary Dysplasia*

The incidence of bronchopulmonary dysplasia (BPD) has increased in recent years due to the increased survival of smaller and smaller premature infants. As a result, BPD now affects at least 10,000 very low-birth-weight (VLBW) infants each year and is associated with neonatal intensive care costs of approximately $30,000–$60,000 per infant. BPD is a multifactorial disease of developmental origin that also involves aspects of inflammation and infection. Therefore, basic research on its etiology is needed to inform meaningful clinical intervention and thereby reduce mortality, morbidity, and the associated high costs of clinical care.

*Brugada's Syndrome*

Translating basic knowledge of the disease into effective therapies remains a challenge.

*Congenital Central Hypoventilation Syndrome*

Locating and recruiting patients remains a problem for congenital central hypoventilation syndrome (CCHS) researchers.

*Congenital Diaphragmatic Hernia*

More basic research is needed on the etiology of congenital diaphragmatic hernia rare disease to reduce mortality and the cost of treating survivors. The average cost of postnatal care exceeds $100,000 with even greater costs incurred for subsequent life care. Because congenital diaphragmatic hernia can now be diagnosed before birth, accurate counseling regarding the expected outcome is crucial.

*Congenital Heart Disease*

With the improvements in surgery made during the past 30 years, many children with congenital heart disease can now grow into adulthood. However, little systematic research has addressed the management of adults with congenital heart disease, and even fewer studies have focused on the

interaction between congenital heart disease and adult cardiovascular diseases. A related concern is that many children need multiple open-heart operations to replace valves that they outgrow. Researchers using bioengineering techniques hope that in the future they will be able to develop valves that would grow with a child. In addition, better understanding of heart development is needed. It is especially important to increase understanding of: (1) the development of coronary arteries, (2) the process by which the heart develops from a symmetric tube to a complex folded structure with a distinct left and right side, and (3) the regulation of heart muscle cell division. The latter especially could be useful in adult cardiology to heal and regenerate damaged myocardium after a heart attack.

*Cooley's Anemia*

Growth and development in patients with thalassemia remains a problem. Cardiac morbidity and mortality in Cooley's anemia (CA) patients is still a major concern. A number of co-morbidities are associated with the chronic transfusions required by people with CA, including infectious diseases (e.g., HIV and hepatitis C) and iron overload. The nature of iron toxicities and their tissue specificities require further study and new approaches to chelation therapy are needed. The morbidity and mortality associated with transplantation of hematopoietic stem cells remain unacceptably high. The potential for gene therapy remains untapped and untested in clinical studies.

*Creutzfeldt-Jakob Disease*

During the past year, the British Government reported the first two cases of possible transmission of variant Creutzfeldt-Jakob disease (vCJD) in transfusion recipients, the first two reports suggesting that the disease might be transmitted to people through blood transfusion. The findings support previous studies of TSE agents in laboratory animals that showed the agents to be present in blood, but in such low concentrations that current tests are not sensitive enough to detect their presence. The potential for blood transmission of vCJD points to the continued need for vigilance by governments, blood and plasma collectors, and manufacturers and underscores the need for a sensitive screening assay to detect vCJD.

*Fanconi Anemia*

Defects in any one of no less than eight different genes can cause Fanconi anemia (FA). The process of identifying the mutation responsible for a particular case of FA is complicated and cumbersome because each of the eight potential genes must be examined for mutations.

*Hemophilia*

Current hemophilia replacement factor therapy requires intravenous infusions that are difficult for small children and may not provide optimal clinical outcomes. New therapeutic approaches are needed to provide a continual supply of the missing factor. Approximately 20 percent of severe hemophilia patients develop antibody inhibitors that specifically neutralize the activity of the replacement factor (e.g., factor VIII) and complicate treatment. The adult hemophilia population

has been severely affected by blood-borne infectious agents in plasma-derived replacement products. Over 80 percent have been infected with hepatitis virus and approximately 20 percent are infected with HIV.

*Hereditary Hemorrhagic Telangiectasia*

Diagnosis of patients with vascular malformations, particularly at an early age, is difficult because multiple organs are affected. Establishment of a genetic linkage may allow earlier diagnosis and improved treatment.

*Homozygous Familial Hypercholesterolemia*

Very few people have homozygous familial hypercholesterolemia, making it difficult to study.

*Idiopathic Pulmonary Fibrosis*

Because idiopathic pulmonary fibrosis (IPF) is a rare disease, it is difficult to recruit enough patients for study. All clinical trials require a multicenter effort. To address this problem the NHLBI has established a clinical research network. To facilitate recruitment of patients, information regarding the NHLBI's intramural trials has been placed on the NHLBI patient recruitment Web site. In addition to difficulties with recruitment, the natural course of IPF is poorly understood and no animal models exactly replicate the human disease. Only one potentially effective treatment, lung transplantation, exists.

*Klippel-Trenaunay-Weber Syndrome*

Since very few people have Klippel-Trenaunay-Weber syndrome (KTWS), it is difficult to recruit patients for studies.

*Long QT Syndrome*

The lack of sufficient numbers of patients with distinct, well-defined, Long QT (LQT) mutations limits researchers' ability to perform clinical studies of interventions. In addition, investigators are working to increase the visibility of the LQTS registry in the African-American medical community. Currently, researchers do not know whether LQTS is less common among African Americans or if they are referred to the registry with less frequency compared to the Caucasian population.

*Lymphangioleiomyomatosis*

Lymphangioleiomyomatosis (LAM) tissue is scarce and cell lines are difficult to establish and maintain. In addition, more work is needed to establish relevant animal models of LAM.

*Lymphedema*

Biotech and pharmaceutical companies are reluctant to invest in research on lymphedema due to a general lack of interest among industries in diseases that do not guarantee a reasonable return on investment.

*Narcolepsy*

Because narcolepsy is a rare disorder, it is difficult to locate and recruit patients for clinical studies.

*Paroxysmal Nocturnal Hemoglobinuria*

Researchers are still working on elements of the eciluzumab clinical trial protocol. More work is needed to determine the selective advantage of paroxysmal nocturnal hemoglobinuria (PHN) cells in the immune environment.

*Persistent Pulmonary Hypertension of the Newborn*

Even with complex and high-risk interventions such as extracorporeal membrane oxygenation, persistent pulmonary hypertension of the newborn (PPHN) currently results in substantial mortality and morbidity.

*Primary Pulmonary Hypertension*

Additional clinical trials are needed to determine the benefits and risks of the various therapies that have recently become available for treating primary pulmonary hypertension (PPH) and the role of combination therapy approaches to treating severe pulmonary hypertension. Because PPH is a rare disease, collaborative efforts are required in order to conduct meaningful clinical trials. Another issue is lack of access to the site of the disease, the lung blood vessels, which has severely limited the ability to study PAH at the cellular and molecular level in humans. Noninvasive methods and biomarkers to monitor pulmonary artery pressure and the course of PPH are needed.

*Sarcoidosis*

Human tissue and animal models are needed to advance basic research on sarcoidosis. In spite of efforts by many researchers over the last half century, no etiologic agent has been identified. Better diagnostic markers and better treatments are needed. Recruiting suitable patients for clinical trials remains a problem area in sarcoidosis research.

*Sickle Cell Disease*

In spite of advances that have been made, several significant therapeutic and psychosocial needs for sickle cell patients remain unmet. Although bone marrow transplantation is available for approximately 20 percent of children with a matched sibling donor, the immediate prospect for a universal cure is still illusive. An understanding of the genetic factors responsible for the

phenotypic differences in sickle cell disease patients' responsiveness to infections and the wide variation in overall clinical severity is needed.

An optimized strategy of pain management for sickle crises is lacking. Prevention and management of undesirable sequealae of chronic transfusions including iron overload is needed. Exploration of psychosocial issues including transition programs from pediatric to adult care, attainment of education goals, and job retention are urgently needed.

*Supravalvular Aortic Stenosis*

Very few people have supravalvular aortic stenosis (SVAS), making it difficult to study.

*Systemic Lupus Erythematosus*

The fear of miscarriage is a great concern for pregnant women with systemic lupus erythematosus (SLE). Therapy to prevent blood clots (anticoagulation) in high-risk pregnant women with antibodies to phospholipids needs evaluation. Trials of more focused and effective anti-inflammatory therapy are also needed. More research is needed to elucidate the factors contributing to accelerated cardiovascular disease in patients with SLE.

*Thrombotic Thrombocytopenic Purpura*

Plasmapheresis has improved the survival of patients with thrombotic thrombocytopenic purpura (TTP), but it remains an expensive and laborious procedure. The metalloprotease ADAMTS 13 could be useful in the development of therapy for familial TTP. The expression and yield of recombinant ADAMTS 13 have limited large-scale production of the protease, which will be needed for clinical studies. A rapid and reliable assay suitable for clinical use to measure ADAMTS 13 activity is needed.

## NATIONAL HUMAN GENOME RESEARCH INSTITUTE (NHGRI)

### Overview of Rare Diseases Research Activities

The National Human Genome Research Institute (NHGRI) led the National Institutes of Health's (NIH) contribution to the International Human Genome Project (HGP). With the achievement of its final goal, the finished sequence of the human genome in April 2003, this project was successfully completed ahead of schedule and under budget and has already begun to change the way we address rare diseases.

In October 2004, the International Human Genome Sequencing Consortium, led in the United States by the NHGRI and the Department of Energy, published an analysis of that finished human genome sequence in the journal *Nature*. This analysis reduces the estimate of the number of human protein-coding genes from 35,000 to only 20,000–25,000—a surprisingly low number for our species, considering that only a decade ago most scientists thought we had over 100,000 genes.

The NHGRI has moved forward into the genomic era with a wide range of powerful new extramural research initiatives that will accelerate genome research and its application to human health. As well, in its Division of Intramural Research (DIR) scientists are using the techniques and tools produced by the HGP and developing new ones to study the fundamental mechanisms of inherited and acquired genetic disorders, including many rare disorders, to lead ultimately to improved diagnostic, prevention, and treatment strategies.

### Inherited Disorders of the Immune System

The NHGRI Genetics and Molecular Biology Branch is conducting a research program to find the causes and develop better treatments for inherited disorders of the immune system. These include immunodeficiencies, in which gene defects impair the ability of the immune system to fight infections, and disorders of immune cell regulation, in which autoimmunity may be seen. Current areas of investigation include severe combined immunodeficiency, hyper-IgE syndrome, certain inherited autoimmune diseases including variants of autoimmune lymphoproliferative disease, and genetic determinants of susceptibility to HIV/AIDS.

*Severe Combined Immunodeficiency*

This project investigates diagnosis and treatment of severe combined immunodeficiency (SCID). Affected infants have severe infections that are fatal unless the immune system can be restored. Bone marrow transplant (BMT) is life-saving if the disease is detected in time. SCID is most often caused by defects in the X-linked IL2RG gene that encodes the common gamma chain of receptors for cytokines. When this gene is defective, lymphocytes do not develop normally. To know how mutations in the IL2RG gene cause X-linked SCID (XSCID), researchers collect samples of blood or tissue, perform DNA analysis, assess expression of common gamma chain protein, and analyze its function. NHGRI researchers can then perform carrier testing and genetic counseling prenatal diagnosis, making affected infants eligible for improved early treatments.

Despite improved survival with BMTs, many XSCID patients are not completely cured, raising the question whether retroviral gene transfer *ex vivo* to white blood cells could improve patients' outcomes. NHGRI researchers have a complete XSCID gene therapy program, including vector development, animal models, retroviral transduction optimization, and clinical evaluation of patients who have failed standard BMT treatment, and are treating patients with a clinical gene therapy protocol.

*Hyper IgE Syndrome (Job Syndrome)*

Hyper-IgE syndrome, also known as Job syndrome after the biblical character who was stricken with boils, is a rare primary immunodeficiency characterized by recurrent skin abscesses, recurrent pneumonia with development of lung cysts, and extreme elevations of serum IgE. The specific immune defect has not been discovered, and NHGRI researchers have undertaken genetic studies to map the disease.

*Autoimmune Lymphoproliferative Syndrome*

Autoimmune lymphoproliferative syndrome (ALPS) is a rare syndrome in which patients have large lymph nodes and spleens, increased numbers of a rare type of lymphocyte called CD4-/CD8- T cells, or double-negative T cells, and defects in programmed cell death, or apoptosis of their lymphocytes. NHGRI researchers have found that most patients with ALPS have inherited mutations in the apoptosis mediator Fas. These patients' lymphocytes do not die when they should; instead, they accumulate and can attack the body's own tissues. Autoimmune diseases of the red blood cells, platelets, and white blood cells are common in ALPS. NHGRI researchers have found some affected members of ALPS families who are more severely affected than others. Modifying genes that influence severity of ALPS are being sought. Researchers have also found that some patients with no mutation in the Fas gene have defects in related genes such as caspase-10

**Developmental Disorders**

Hutchinson-Gilford progeria syndrome (HGPS) is the most dramatic human syndrome of premature aging. Children with this rare condition are normal at birth, but by age 2 they have stopped growing, lost their hair, and shown skin changes and loss of subcutaneous tissue that resemble the ravages of old age. They rarely live past adolescence, dying almost always of advanced cardiovascular disease (heart attack and stroke). Using genome-wide scans, NHGRI researchers identified an area on chromosome 1q that was a candidate for HGPS mutations in a subset of patients. After sequencing a plausible candidate gene in the suggested area, the gene for lamin A/C (LMNA), the researchers discovered that nearly all cases of HGPS harbor a de novo point mutation in the LMNA gene. Researchers found that the mutation creates a smaller, truncated version of the lamin A/C protein, which is now referred to as progerin. The researchers have shown that progerin acts in a dominant manner to disrupt the structure of the cell’s nuclear membrane scaffold.

Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE, Gordon LB, Gruenbaum Y, Khuon S, Mendez M, Varga R, Collins FS (2004) Accumulation of mutant lamin A causes

progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci U S A.* 101:8963-8. [Pubmed]

*Polydactyly Syndromes*

A group of syndromes that include polydactyly with other malformations is the subject of a clinical-molecular study. This research study encompasses a range of phenotypes that include Pallister-Hall syndrome, the allelic disorder Greig cephalopolysyndactyly syndrome (GCPS), McKusick-Kaufman syndrome (MKS), and Bardet-Biedl syndrome (BBS). The clinical manifestations of these disorders include polydactyly, central nervous system malformations (with or without mental retardation and seizures), craniofacial malformations, and visceral malformations such as renal malformations or congenital heart defects. NHGRI researchers study these disorders by a translational approach that begins in the clinic with careful clinical evaluation of the phenotypes by physical examination and imaging studies that include radiographs, ultrasound, MRI, and CT scanning. They have shown that BBS and MKS can both be caused by mutations in the same gene. PHS and GCPS are caused by a wide spectrum of mutations in the GLI3 gene. The severity of the GCPS phenotype, specifically the mental retardation and learning disability, are correlated with these mutations. Patients with larger deletions have a more severe form of the disease. Researchers are also characterizing a mouse mutant that also has phenocopy of the extra toes in a Gli3 mouse mutant but is linked to a gene other than Gli3. This animal could shed light on other genes in the Gli3 pathway.

*Lowe Oculocerebrorenal Syndrome*

The oculocerebrorenal syndrome of Lowe is a rare X-linked metabolic disorder characterized by congenital cataracts, renal tubular dysfunction, and mental retardation. It is caused by mutations in the gene OCRL1 encoding a specific phosphatase. Research at NHGRI has resulted in the identification of the responsible gene, determination of its biochemical function, and the development of accurate enzymatic diagnosis for affected fetuses and individuals. The current focus of this research is to understand how a defect in this enzyme results in the various manifestations of the syndrome. Researchers are working both in cultured human cells and in animal models. They have demonstrated that the cell structural skeleton is disorganized in cultured cells from Lowe syndrome patients. They are investigating the role of intracellular calcium in bringing about this phenotype and have found abnormal calcium signaling in patient cells. Researchers are also working to create a mouse model for Lowe syndrome.

*Hirschsprung Disease*

Animals heterozygous for mutations in the SOX10 transcription factor exhibit multiple defects in neural crest development, including reduced numbers of melanocytes in the skin, an absence of myenteric ganglion in the colon, and deafness. A human congenital disorder, Hirschsprung disease, also exhibits rectocolic aganglionosis and can be associated with hypopigmentation caused by SOX10 mutations. Thus SOX10 mice, as well as other neural crest mutant mice, serve as mouse models for this disease. Investigation of the involvement of SOX10 in Hirschsprung disease and other neural crest-related disorders is being explored. NHGRI researchers have established a system for adding genes back to neural crest stem cells in order to complement

genetic defects. They plan to use this system to test hierarchial relationships between SOX10 and its target genes and have demonstrated that they can use this system to correct SOX10 defects *in vitro*.

*Microphthalmia Syndromes*

The project seeks to understand the clinical and molecular basis of syndromic microphthalmia. This disorder comprises anophthalmia or microphthalmia (small or absent eyes with blindness), mental retardation, and skeletal anomalies. NHGRI researchers have identified a large family affected by Lenz microphthalmia and have mapped the gene to the short arm of the X chromosome. This result is surprising because another family with this disorder maps to the long arm of the X chromosome. This means that Lenz microphthalmia is probably an amalgam of two disorders. Researchers have used positional cloning to isolate the gene that is altered in the condition, which is called BCOR (BCL-6 co-repressor). In addition, researchers have discovered that mutations in this gene also cause the Oculo-facio-cardi-dental syndrome. They are currently assessing the functional consequence of these mutations in a zebrafish model system. The results of this research should allow development of accurate diagnostic tests for microphthalmia and improved understanding of eye development

*Molecular Genetics of Anabaptist Diseases*

Old Order Amish and related Anabaptist sects (including Mennonites) are important for the study of genetic disease as they represent a cultural and genetic population isolate. In addition, they are enthusiastic historians and have excellent printed genealogical records. NHGRI researchers have built the Amish Genealogy Database (AGDB) and several computational tools to analyze the database including PedHunter. These tools allow generation of pedigrees for genetic study in an accurate and rapid fashion. NHGRI researchers have also cloned the genes that are altered in glycogen storage disease type 6, McKusick-Kaufman syndrome, and Amish nemaline myopathy and are currently working on Amish microcephaly. They have identified the gene alteration in Amish microcephaly that codes for a protein that transports deoxynucleotides (DNA precursors) into the mitochondria. They have currently developed both mouse and zebrafish animal models for this disorder and are now characterizing the phenotypes of these organisms.

*Proteus Syndrome*

Proteus syndrome (PS) is a rare, sporadic syndrome that causes progressive, patchy overgrowth, bony distortion or deformation, tumor predisposition, and mental retardation. NHGRI researchers are determining the natural history and etiology of Proteus syndrome. The natural history and the phenotypic range are being determined by clinical assessment and longitudinal follow-up of a cohort of patients. Very little is known about the natural history and the range of the phenotype of PS. NHGRI scientists are accruing a cohort of patients with PS and overlapping phenotypes and plan to follow them over time. As the disorder is usually apparent at or soon after birth and appears to evolve at least into the 20s, it will be necessary to have long-term follow-up. The etiology of PS has been studied using various comparative molecular biology techniques including representational difference analysis, cDNA arrays, and other techniques. Researchers are currently in the process of analyzing expression array data. The researchers have also performed a

retrospective literature review and reclassified all previously published patients according to their new clinical criteria. This analysis shows that the lethality of the disorder is higher than previously believed, affects more males than females, and has a high rate of complications.

*Alagille Syndrome*

Researchers at NHGRI have shown that mutations in the Jagged1 (JAG1) gene are responsible for Alagille syndrome (AGS), a developmental disorder affecting multiple organ systems including liver, heart, eye, face, and vertebrae. Zebrafish is an excellent model for vertebrate development, and therefore researchers have initiated efforts to explore the role of jagged genes in zebrafish development and in developmental diseases like AGS. As a part of this effort, they have isolated and characterized jagged homologous genes from zebrafish.

*Congenital Disorders of Glycosylation*

Congenital disorders of glycosylation (CDG) are a diverse group of metabolic disorders presenting with a spectrum of clinical features ranging from severe neurologic manifestations and multisystemic involvement to hypoglycemia and severe gastrointestinal symptoms with normal development. There are now 16 types of CDG defined by distinct enzyme defects and genes. The number of children and adults diagnosed with CDG in the United States is increasing rapidly with a wider variance in the phenotypes. NHGRI researchers will identify and evaluate individual patients with CDG, explore the clinical and biochemical features of untyped individuals and, through clinical research, continue to add to the compendium of clinical management strategies for physicians caring for these affected adults and children.

**Neurological Disorders**

*Familial Encephalopathy with Neuronal Inclusion Bodies*

NHGRI researchers are exploring the clinical, laboratory, neuropsyche, and imaging features of at-risk members of families with familial presenile dementia with neuroserpin storage. Over the past year the name of the disorder has been changed to familial encephalopathy with neuronal inclusion bodies (FENIB). At the NIH Clinical Center researchers have seen 25 individuals at risk for this disorder for full clinical evaluations. The clinical facet of this project continues to be a long-term exploration of the natural history of family members at risk. This will increase insight into the pathophysiology and clinical presentation of FENIB. These families provide not only rich clinical insight but the opportunity to understand the controversy surrounding presymptomatic testing in late-onset neurodegenerative disorders. Further clinical delineation and assessment of counselling needs remain the clinical goals for this project. A parallel laboratory project includes the development of a mouse model for FENIB that will further help to elucidate the phenotype and genotypic variation.

*Huntington's Disease*

Research suggests that appraisals of a stressful event, such as the likelihood of change and perceived ability to cope, are important predictors of the use of coping strategies and overall

adaptation. This NHGRI study explores the relationships between coping self-efficacy, coping strategies, and grief reaction with individuals who have undergone presymptomatic genetic testing for Huntington's disease (HD). Eligibility criteria included individuals who tested negative or positive and who were currently without symptoms of HD. Measures included the Coping Self-Efficacy Scale, the Ways of Coping Checklist-Revised, and the Grief Experience Inventory. Preliminary regression analysis revealed coping self-efficacy to be negatively associated with the use of avoidance coping and avoidance coping to be positively associated with grief scores. Respondents showed greater use of problem-focused coping strategies compared to emotion-focused strategies. Problem-focused coping strategies were negatively associated with measures of grief, while emotion-focused coping was positively associated with grief scores. An individual's coping self-efficacy predicts the use of particular coping strategies that in turn can predict the degree of grief experienced. On the basis of this work, genetic counseling interventions have been proposed to maximize coping self-efficacy and effective coping strategies in light of a genetic test result for a condition that has no treatment or cure.

**Endocrine Disorders**

*Multiple Endocrine Neoplasia Type 1*

Multiple endocrine neoplasia type 1 (MEN1) is characterized by multiple tumors of the parathyroid, anterior pituitary, and GI endocrine tissues. NHGRI researchers have previously shown that mutations in the MEN1 gene are responsible for the MEN1 syndrome. The MEN1 encoded nuclear protein, Menin, binds the transcription factors JunD and NFκB and can repress JunD and NFκB-induced transcription. By expressing wild-type or mutant JunD in mouse fibroblast cell lines that do not express menin and JunD, NHGRI researchers found that interaction with menin is required for the growth suppressor function(s) of JunD. They have developed both conventional and conditional mouse knockout models that yield phenotypes that are remarkably similar to the human MEN1 disease and have allowed delineation of the stages in tumor development. Researchers have developed tissue-specific menin-inducible transgenic mouse models and are currently creating a drosophila model.

**Disorders of Vision**

*Rieger Syndrome*

A continuing area of interest of researchers at NHGRI involves the homeodomain family of proteins, which play a fundamental role in a diverse set of functions that include body plan specification, pattern formation, and cell fate determination during metazoan development. Members of this family are characterized by a helix-turn-helix DNA-binding motif known as the homeodomain. Homeodomain proteins regulate various cellular processes by specifically binding to the transcriptional control region of a target gene.

These proteins have been conserved across a diverse range of species, from yeast to human. A number of inherited human disorders are caused by mutations in homeodomain-containing proteins. One specific homeodomain protein, FOXC1, is implicated in Axenfeld-Rieger malformations. Patients with Axenfeld-Rieger malformations typically show a spectrum of ocular

findings, including iris hypoplasia, a prominent Schwalbe line, iris adhesions, and goniodysgenesis. The most severe cases show elevated intraocular pressure, leading to the development of glaucoma. As an outgrowth of the studies on the homeodomain class of proteins, NHGRI researchers have developed and continue to maintain the Homeodomain Resource. This publicly available database provides a curated collection of information that includes full-length homeodomain-containing sequence data, experimentally derived structures, protein-protein interaction data, DNA-binding sites, and mutations leading to human genetic disorders. Work is continuing in this area of homeodomain proteins to better understand these eye-related mutations and their net effect on vision.

**Metabolic Disorders**

*Methylmalonic Acidemia and Related Disorders*

Methylmalonic acidemia (MMA) is a genetically heterogeneous disorder of methylmalonate and cobalamin (vitamin B12) metabolism. Symptoms of MMA usually begin in the first few months of life and include lethargy, failure to thrive, vomiting, dehydration, respiratory distress, hypotonia, and hepatomegaly. Acute episodes may include drowsiness, coma, and seizures, with subsequent developmental delays. An NHGRI research study encompasses the hereditary methylmalonic acidemias and cobalamin deficiency disorders. These metabolic disorders are genetically heterogeneous and collectively represent an important subset of the organic acidemias. The general goal of the research is to define the complications seen in patients, replicate the findings in mice or other organisms, and use the combined information to guide the development and testing of new therapies. Researchers study the hereditary methylmalonic acidemias and cobalamin deficiency disorders via a translational approach that includes a clinical and metabolic evaluation of affected patients and use animal models to examine the disorder in the laboratory. In addition, they have developed mouse and worm models of methylmalonic acidemia.

**Additional Activities**

*Genetic and Rare Diseases Information Center*

In order to respond to the public's need for information on genetic and rare disorders, NHGRI and the Office of Rare Diseases, NIH, maintains and supports the NHGRI/ORD Genetic and Rare Diseases Information Center (GARD). The Information Center focuses on meeting the information needs of the general public, including patients and their families, health care professionals, and biomedical researchers. The purposes of the Information Center are to: (1) serve as a central, national repository of information materials and resources on genetic and rare diseases, conditions, and disorders; (2) collect, produce, update, and disseminate information on the diagnosis, treatment, and prevention of genetic and rare disorders; and (3) coordinate with organizations and associations interested in genetic and rare disorders to explore networking capabilities, avoid duplication of effort, and identify information gaps. See: http://rarediseases.info.nih.gov/html/resources/info_cntr.html

## NATIONAL INSTITUTE OF MENTAL HEALTH (NIMH)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Mental Health (NIMH) is to reduce the burden of mental illness and behavioral disorders through research on mind, brain, and behavior. As reported in the World Health Organization's Global Burden of Disease study, mental disorders comprise four of the top five sources of premature death and disability in 15–44 year olds in the Western world. Serious mental illnesses such as schizophrenia, depression, bipolar disorder, anxiety disorders, and autism are the primary foci of research that NIMH supports and conducts. Additional research areas of significance to NIMH that can be classified as rare diseases include childhood-onset schizophrenia; pediatric bipolar disorder; Gaucher disease; William's syndrome; Klinefelter's syndrome; suicide; and pediatric and geriatric HIV/AIDS.

### Recent Scientific Advances in Rare Diseases Research

*Childhood-onset Schizophrenia*

Childhood schizophrenia, defined as onset of psychosis by age 12, is a severe and unremitting form of the disorder. Patients with this rare, severe illness have profound impairment in development and resemble adult patients with poor-outcome schizophrenia. Several advances have been made in the study of childhood-onset schizophrenia (COS) this year at NIMH and further help elucidate the genetic vulnerabilities for the disorder.

Recent brain imaging studies have shown that COS is associated with striking progressive loss of cerebral volume during adolescence, but it was not known if these changes were specific to COS. MRI studies were used to compare COS patients with pediatric patients with transient psychosis and behavioral problems; both patient groups had similar early developmental patterns, cognitive functioning, medications, and hospitalizations. The results showed that the gray matter loss only occurred in the COS patients, which suggests that this loss is related to the illness and not related to medication. Ongoing MRI studies of siblings of COS patients will address whether this abnormal development may be a trait marker for the disease.

Although the above study showed distinct differences between COS and other childhood-onset psychotic disorders, there may be some genetic similarities between COS and bipolar disorder. The genetic component of COS was investigated in a study that looked at the relationship between two overlapping genes, G72 and G30, which have both been shown to be involved in the susceptibility to schizophrenia and bipolar disorder in adult patients. The study, which used patients diagnosed with COS or psychosis not otherwise specified, found significant associations between genetic markers at the G72/G30 locus and childhood-onset psychotic illness. It also found an association between the G72/G30 locus and the age of onset of psychotic symptoms, which was found to be close to adolescence. These findings confirm and strengthen previous reports that G72/G30 is a susceptibility locus both for schizophrenia and bipolar disorder.

Family, twin, and adoption studies have demonstrated that the development of schizophrenia is heavily influenced by genetic components. Studies have also shown that the synthesis and function of the neurotransmitter GABA may be affected in schizophrenia. (Postmortem brain studies have shown deficits in the cortical GABA system of schizophrenic individuals compared to controls, including a decrease in the production of the major GABA-synthesizing enzyme glutamic acid decarboxylase [$GAD_{67}$]. Genetic analysis of a group of children and adolescents with COS was used to explore a possible association between COS and alterations in the genes coding for $GAD_{67}$, an enzyme involved in the synthesis of GABA. The results showed that there was a positive association between COS and specific variations in the genes encoding $GAD_{67}$; these alterations in the gene encoding $GAD_{67}$ could potentially prove to be a fairly common risk factor for schizophrenia.

*Pediatric Bipolar Disorder*

NIMH researchers are currently developing neuropsychological tests that would help investigate pediatric bipolar disorder. Existing neuropsychological tests for children with bipolar disorder are scarce and can only be used to investigate cognitive function, leaving the affective domain largely unexplored. Children with bipolar disorder exhibit affective symptoms that reflect an abnormal experience of pleasure. During depressive episodes these children exhibit an inability to seek or experience pleasure, whereas during manic episodes, pleasure-seeking behavior is heightened. In the first experimental study to examine associations between pediatric bipolar disorder and reward-related behaviors, reward behaviors in bipolar and control children were examined using a Wheel of Fortune task in which subjects could win or lose money based upon their decisions. The results showed that while bipolar children do not make risky choices more often than control children, they have significantly less confidence in favorable outcomes and a more emotional response to feedback. The ultimate goal of this work is to eventually use this task in conjunction with fMRI imaging to study reward pathways in pediatric bipolar disorder.

*Gaucher Disease*

Gaucher disease (GD) is a rare, inherited metabolic disorder in which deficiency of the enzyme glucocerebrosidase results in the accumulation of harmful quantities of certain lipids throughout the body, particularly within the bone marrow, spleen, and liver. The symptoms associated with GD vary greatly. There are three general classifications of GD, each defined by the extent of neurological complications. Type 1 GD is the most common form and involves essentially the peripheral organs and has no primary neuropathy. The onset of neuropathy begins in Type 2 GD and becomes chronic at Type 3. In order to understand this progressive neuropathy, NIMH researchers sought to identify specific patterns of neuronal injury associated with GD. Unique pathologic patterns were found in two specific regions of the brain, the hippocampus and calcarine cortex. The pathologic patterns were found in all three types of GD and the extent of these structural changes varied with the stage of the disease. This work suggests that GD, Parkinson's disease, and diffuse Lewy body dementia (a condition with symptoms similar to those seen in Alzheimer's or Parkinson's disease that involves a specific pattern of neuronal loss, i.e., globe-shaped structures or lesions present in the nerve cells of the brainstem and cortical brain areas that cause symptoms similar to those seen in Alzheimer's or Parkinson's disease) have common cytotoxic pathogenic mechanisms affecting the cells of the nervous system.

*Williams Syndrome*

The genetic basis of Williams syndrome (WS) is remarkably well understood; it is caused by a hemizygous deletion of approximately 21 genes on chromosome 7. Disturbed visuospatial construction is a hallmark of the disorder and individuals with WS have a number of cognitive deficits, including mental retardation and learning disabilities. A hallmark of the disorder is a marked difficulty in visualizing an object as a set of parts and constructing a replica (e.g., assembling a puzzle or constructing a piece of furniture from separate parts). This deficit is referred to as disturbed visuospatial construction. People with WS also tend to be overly gregarious and anxious and often have mental retardation and learning disabilities. Scientists at NIMH used neuroimaging to trace the thinking deficit to underactivation of a circuit in the back of the brain that processes locations of objects in the visual field as compared to controls. Further analysis revealed that the nonfunctional and adjacent area of the brain that feeds information to the underactive areas was structurally altered in people with WS. This research confirms a long-standing hypothesis explaining disturbed visuospatial construction in WS and demonstrates the effects of a localized brain abnormality on visual information processing in humans. Finally, because the genetic basis of WS is so well understood, this study demonstrates ways in which known genetic abnormalities can lead to alterations in brain organization and function and, ultimately, to cognitive deficits. These structurally altered areas may also be important in other pathophysiological mechanisms in WS (structural neuroimaging showed a gray matter volume reduction in three brain regions of participants with WS) in addition to the cognitive deficit in visuospatial construction. This work demonstrates the effects of a localized abnormality on visual information processing in humans and defines a systems-level phenotype for mapping genetic determinants of visuoconstructive function.

*Klinefelter's Syndrome*

XXY chromosome arrangement, instead of the usual male arrangement (XY), appears to be one of the most common genetic abnormalities known. The presence of an extra X chromosome is often related to atypical physical, cognitive, and behavioral features. NIMH researchers used fMRI to investigate changes in brain morphometry associated with Klinefelter's syndrome. The results showed that compared to XY controls, XXY males exhibit pronounced brain volume reduction, which was localized in specific areas of the brain—the amygdala, hippocampus, insula, temporal gyri, cingulate, and occipital gyri. In addition to the reduction in volume in these specific areas, the results also found overall enlargement of ventricles and overall volume reduction of both white and gray matter in XXY males. Based on these results, future experiments will be designed to understand the behavioral correlates of the structurally altered regions.

**Rare Disease-specific Request for Applications**

*HIV/AIDS and Aging*

Following a meeting supported by ORD in April 2001 ("Mental Health Research Issues in HIV Infection and Aging"), RFA MH03-004 ("HIV/AIDS and Aging: Basic and Clinical Research") was released. The following five grants were funded from this RFA, including projects in three basic research areas, clinical neuroAIDS, and clinical intervention:

(1) Haughey, Norman J; HIV-Associated CNS Dysfunction with Aging; R01 MH069177-01, R01 AG23471-01;
(2) Johnson, Rodney W; Aging, Brain Cytokines and HIV-Associated Dementia; R01 MH069148-01;
(3) Shiramizu, Bruce T; Activated Immune Parameters Associated with HIV and Aging; R21 MH069173-01;
(4) Gelman, Benjamin B; HIV and the Proteasome in the Aging Human Brain; R01 MH069200-01;
(5) Camp, Cameron J; HIV/AIDS and Aging: A Cognitive Clinical Intervention; R21 MH069199-01.

These studies will provide the foundation for further work focused on basic and clinical science aspects of HIV and aging, including increased emphasis on preventive interventions and their application internationally. It is hoped that this information will provide a foundation for expanding our efforts on the study of HIV and aging, with additional concentration on preventive interventions and their application in the international arena.

**Significant Ongoing Rare Diseases Research Initiatives**

*Suicide*

Important ongoing work on suicide is being conducted through two NIMH projects that have received ORD supplementary support. One study on high-risk delinquents and young adults continues to assess suicidal behavior, noting a potential for the under-reporting of suicidal behavior in African-American subjects. The other study has made use of supplemental funding from the ORD to develop a methodology for assessing population-based approaches to the prevention of suicide. In FY 2002, ORD awarded two supplements to NIMH grantees, who then produced products from those awards in FY 2004. Dr. Linda Teplin of Northwestern University (MH5943) received monies to support assessment of suicidal behavior in high-risk delinquents and young adults. Her progress report (forthcoming to ORD) will include a summary of violent deaths in this cohort and raises the question of whether suicides in African-American males in her study cohort are underestimated. Dr. Hendricks Brown of the University of South Florida also received a supplement to develop a methodology for population-based approaches to the prevention of suicide. His progress report (also forthcoming to ORD) will describe how data can be combined across prevention trials through a meta-analytic approach to have sufficient power to detect

preventive intervention effects for suicide deaths as well as steps necessary to address measurement error in death records.

In other work on suicide, an ongoing NIMH-funded randomized controlled trial is investigating the efficacy of a Youth-nominated Support Team Intervention (YST) that targets treatment adherence and family/social support among 13- to 17-year-old suicidal adolescents during the high-risk period following psychiatric hospitalization. The addition of the YST intervention to usual care is expected to result in reductions in psychiatric symptom severity and suicide attempts/ideation as well as improvements in treatment adherence, social support, and adaptive functioning. Results of this study will address an important gap in our understanding of strategies for improving treatment adherence and reducing suicide risk among these adolescents.

Several additional Career Awards focused on psychosocial and pharmacologic interventions for suicidal adolescents will help address a critical need for more research capacity in this area. These initiatives included awards to established investigators to support their efforts at mentoring new investigators and facilitating their development as independent investigators and an early career award to an investigator who is studying the feasibility and preliminary efficacy of cognitive-behavior therapy and pharmacotherapy for decreasing adolescents' depressive symptoms and suicidal behaviors.

*Pediatric HIV/AIDS*

Following a meeting supported by ORD in 2001, a study investigating the prevalence of psychiatric symptomatology—particularly focusing on attention deficit disorders in youth perinatally infected with or exposed to HIV-1—was designed. NIMH partnered with NICHD and NIAID to fund this study, conducted in cooperation with the Pediatric AIDS Clinical Trial Group (PACTG), and a PACTG protocol is in the final phases of approval. The PACTG is the largest clinical research program in the United States focused on the care and treatment of HIV-infected children and is funded by NIAID and NICHD. NIMH will fund this substudy to identify, quantify, and characterize the psychiatric consequences of living with HIV from birth. The goal is to develop an understanding of the mediating and moderating factors that lead to abnormalities and to ultimately develop interventions to prevent or lessen the psychiatric consequences of living with HIV in children and adolescents. The study will include both HIV-1-infected and uninfected but exposed children, and the impact of type and duration of antiretroviral treatment will be evaluated. This information will become of great importance in developing countries as antiretroviral therapy becomes more available and children and adults live longer.

**Rare Disease-specific Conferences, Symposia, and Meetings**

*Suicide*

In October 2003, NIMH with cofunding from the ORD, CDC, SAMHSA, and the Annenberg Foundation held a workshop titled "The Science of Public Messages for Suicide Prevention." No evaluations of public awareness campaigns for suicide prevention have been published, leaving minimal guidance for future efforts to create effective public messages that increase awareness that suicide is preventable. To address "how to" develop and evaluate public awareness campaigns, workshop participants (including suicide prevention advocates and experts in public health evaluation, suicide contagion, decision-making, and marketing) were asked to use logic models to define intended messages and audiences, assumed mechanisms of change, and outcomes. A summary of the meeting has been posted on the NIMH Web site (http://www.nimh.nih.gov/SuicideResearch/SuicidePreventionOct2003.cfm) and a longer summary has been accepted for publication in the journal *Suicide and Life Threatening Behavior*.

On September 9–10, 2004, NIMH with ORD support, the Annenberg Foundation, and Emerging Scholars Interdisciplinary Network cosponsored a workshop titled "Pragmatic Considerations of Culture in Preventing Suicide" at the Annenberg Center in Philadelphia. U.S. suicide rate patterns associated with age, gender, and ethnicity provide profound evidence that culture is associated with suicide risk and protective factors. The purpose of the meeting was to examine how culture pertaining to ethnicity can be considered in the development and implementation of suicide prevention interventions. Three fielded studies were considered as examples in reducing suicide risk and included studies focused on African-American, Hispanic/Latino, and American Indian communities. For this workshop, culture was defined as self- and community-identity, community norms, and behavioral practices that can affect how an individual engages in behavior linked to life or death outcomes. Recognizing that this field is in an early stage of development, approaches to theory, measurement, and intervention development for each of the three studies were discussed with regard to their strengths and opportunities for further development. Experts knowledgeable about suicide risk for other understudied minority groups (e.g., Pacific Islanders) were asked to attend and provide feedback on the content and process of the workshop and recommend approaches for future meetings. A summary of the meeting has been drafted for the Web and has been submitted for clearance. Recommendations from this meeting are being used for planning a conference on suicide prevention among indigenous peoples in the Americas.

## NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE (NINDS)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Neurological Disorders and Stroke (NINDS) is to reduce the burden of neurological disease—a burden borne by every age group and every segment of society worldwide. The brain, spinal cord, and nerves are vulnerable to hundreds of disorders, most of which are rare. Even more common diseases such as stroke, epilepsy, and Parkinson's disease include rare subtypes. NINDS supports research to uncover the causes of and develop treatments for individual rare disorders, while also promoting cross-cutting research on topics such as stem cells, gene therapy, and neuroimaging that will impact multiple neurological disorders.

NINDS supports basic, clinical, and translational research on rare diseases through both its extramural and intramural programs. NINDS also collaborates with the NIH Office of Rare Diseases (ORD) and rare disease voluntary organizations to stimulate specific research areas via workshops, grant solicitations, and strategic planning efforts. The Institute's primary support of research is through unsolicited, investigator-initiated grant awards, as investigators often have the greatest insight into the critical questions facing a particular field of research. Of the new grants funded by the NINDS in FY 2004, many focused on rare diseases. Examples include prion diseases, Batten disease, Niemann-Pick C, Huntington's disease, muscular dystrophy, tuberous sclerosis, Rett syndrome, Fragile X, Joubert syndrome, Hurler syndrome, Tay-Sachs disease, Wilson's disease, narcolepsy, Lowe syndrome, sickle cell anemia, Williams syndrome, dystonia, neurofibromatosis, neural tube defects, amyotrophic lateral sclerosis, and Friedreich's ataxia.

### Recent Scientific Advances in Rare Diseases Research

*AtaxiaTelangiectasia*

Ataxia-telangiectasia is a severe neurodegenerative disease that affects the brain and other systems in childhood and is caused by mutations in the ATM (A-T mutated) gene. In about 14 percent of cases, the mutation creates a "stop" signal in the gene, which causes the ATM protein to be truncated and therefore become nonfunctional. Researchers now have determined that certain antibiotics (aminoglycosides) allow cultured cells to ignore this stop sign and make full-length and functional ATM protein. Several clinical studies have shown that a relatively small amount of functional ATM protein can be beneficial, so this study using antibiotics is a promising strategy for follow-up in animal studies and possibly for clinical trials in humans.

*Fabry Disease*

Fabry disease is a hereditary disorder characterized by recurrent episodes of severe pain and other symptoms including skin, heart, eye, and kidney problems. The disease is caused by a faulty enzyme, α-galactosidase, which causes lipids to accumulate in cells to harmful levels. NINDS intramural researchers have made advances toward several therapies for Fabry disease. The FDA has approved an enzyme replacement therapy for Fabry disease based on positive results in NINDS intramural trials. Because enzyme replacement therapy may have limitations for long-

term use, intramural researchers are also exploring gene therapy as a potential treatment for Fabry disease. Following up on successful gene therapy experiments in adult mice, researchers recently showed that a single treatment given to newborn Fabry mice with a virus containing the α-galactoside gene resulted in long-term correction of the disease defect without an undesirable immune response. If the therapy can be adapted to humans, it may provide a well-tolerated treatment for the disease at an early stage before irreversible organ damage occurs.

*Familial Dysautonomia (hereditary sensory and autonomic neuropathy type III)*

Hereditary sensory and autonomic neuropathy type III, also called Riley-Day syndrome or familial dysautonomia, disrupts the brain's control of bodily systems such as gastrointestinal function, respiration, and the cardiovascular system. In people with this disease, a gene mutation causes cells to leave out a critical segment of the IKBAP protein, leading ultimately to degeneration of nerve cells. Researchers have found that kinetin, a plant growth regulating hormone, significantly increases the production of normal IKBAP protein in blood cells that have the disease-causing gene defect. This drug was found by screening a set of 1040 FDA-approved candidate compounds in the NINDS Custom Collection developed for screening against models of neurological disorders. The finding that a drug can increase production of normal full-length IKBAP in cells is exciting because even subtle changes in the level of protein can have a drastic effect on the severity of this and other diseases caused by similar defects.

*Muscular Dystrophy*

Duchenne muscular dystrophy is an inherited disorder that causes progressive degeneration of skeletal muscles, leading to severe disability and, ultimately, death. More than a decade ago, researchers discovered that a genetic mutation leads to lack of a protein called dystrophin, which in turn causes muscle degeneration. Researchers have developed a method to deliver therapeutic genes to skeletal muscles throughout the body of a mouse strain that mimics human Duchenne muscular dystrophy. To carry the corrective gene, a modified version of the dystrophin gene that is smaller but still functional, the investigators used an engineered version of a specific type of virus, which can enter muscle cells and not trigger an immune response. Importantly, the investigators co-injected a blood vessel growth factor that temporarily renders the blood vessels permeable so that the vector can efficiently pass through. The corrective gene was widely expressed throughout the skeletal muscles of the mice, and the dystrophy in the mice was dramatically improved by the procedure.

The investigators are gathering the necessary data to see if this treatment can be safely tried in people, which may still be several years away. Researchers need to know, for example, whether the procedure will work in larger animals and whether it remains safe over longer periods of time. If successful, the strategy might be adapted to carry different genes for treating other muscle diseases, including several other forms of muscular dystrophy, and also to deliver therapeutic genes to heart muscle cells.

*Rapid-Onset Dystonia Parkinsonism*

Researchers have identified the gene defects responsible for a rare inherited form of dystonia, known as rapid-onset dystonia parkinsonism (RDP), that usually strikes adolescents or young adults. RDP is unusual in that symptoms arise very suddenly, often following emotional or physical stress such as fever, childbirth, or prolonged exposure to heat or exercise. The gene defects lead to abnormalities in a protein that normally pumps potassium and sodium into and out of cells to maintain their proper concentrations when nerve cells are active. One possibility is that the abnormal pump protein cannot keep up in stressful circumstances, triggering disease symptoms. Future studies will determine how mutations in the gene cause susceptibility to RDP and might also provide clues to other forms of dystonia, parkinsonism, or even epilepsy.

*Spinal Bulbar Muscular Atrophy*

X-linked spinal bulbar muscular atrophy, also called SBMA or Kennedy's disease, is an inherited disorder that causes motor neurons, the nerve cells that control muscles, to degenerate. The disease affects adult men and causes progressively worsening muscle weakness, cramping, and atrophy, which can also result in problems with speech and swallowing. Scientists have determined a critical link by which the gene mutation in SBMA causes the death of motor neurons. The research team genetically engineered a mouse strain that carries the human mutation and develops symptoms strikingly reminiscent of human SBMA. By studying the mice, the researchers determined that the levels of VEGF, a natural growth-promoting substance, were dramatically reduced in the spinal cord before the onset of symptoms. The reduction of VEGF appeared to result from an interaction between the mutated gene product and a regulator molecule. In a first step toward testing a therapeutic strategy based on this finding, the investigators found that artificially increasing VEGF itself or its regulator rescued motor neuron-like cells in a cell culture model of SBMA. Further experiments are under way in the SBMA mice to test this approach.

*Spinocerebellar Ataxia Type I*

Spinocerebellar ataxia type 1 (SCA1) is one of many neurodegenerative diseases caused by a dominant inherited mechanism, where a single defective copy of a gene from either parent produces a defective protein that is harmful and causes disease. People with spinocerebellar ataxias experience failure of muscle control in their arms and legs, balance problems, and disturbance of gait, which progressively worsens. Researchers have used a promising new therapy to silence the toxic disease gene in mice genetically engineered to carry the gene defect that causes human SCA1. Researchers developed a modified (and harmless) virus that carries a molecule, called a short hairpin RNA. This molecule is designed to suppress only the defective gene through a process called RNA interference. RNA interference takes advantage of a recently discovered process that cells may have evolved to help protect themselves from viruses. Young SCA1 mice treated with this procedure showed strongly improved movement control and a healthier brain structure than untreated mice. If this approach can be made safe and effective for use in people, this new therapeutic strategy should be applicable to other dominantly inherited neurodegenerative diseases.

**New/Planned Research Initiatives**

NINDS supported and stimulated research related to rare disease under the following FY 2004 solicitations, some of which were issued in collaboration with other Institutes and patient voluntary organizations:

- Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers (with NIAMS and NICHD)
- The Etiology, Pathogenesis, and Treatment of ALS (with Department of Veteran's Affairs and the Amyotrophic Lateral Sclerosis Association)
- National Centers for Neurofibromatosis Research (with NIDCD)
- CNS Therapy Development for Lysosomal Storage Disorders (with NIH ORD and the Lysosomal Storage Disease Research Consortium)
- The SMA Project: A Collaborative Program to Accelerate Therapeutics Development for Spinal Muscular Atrophy
- NINDS Pilot Therapeutics Network Clinical Operations Center: NPTUNE COC
- Announcement of Request for Proposals: Inducible Mouse Models of Spinal Muscular Atrophy (SMA)
- Novel Approaches to Enhance Animal Stem Cell Research (with multiple NIH Institutes)

Among planned solicitations are:

- Microarray Centers for Research on the Nervous System (with NIMH)
- Centers of Excellence in Translational Human Stem Cell Research (with NIDDK and NHLBI)
- An *In Vitro* Biological Assay Facility for Screening Compounds in Cellular Models of Spinal Muscular Atrophy (SMA)
- NIH Administrative Supplements for Senator Paul D. Wellstone Muscular Dystrophy Research Fellowships at Wellstone Muscular Dystrophy Cooperative Research Centers (MDCRCs)
- Accelerating Therapy Development for Tuberous Sclerosis
- Basic and Clinical Research on Rett Syndrome and MeCP2
- Delivery of RNAi Therapeutics into the Nervous System
- Mechanisms of Transmission and Dissemination of Transmissible Spongiform Encephalopathies

**Significant Ongoing Rare Disease Research Activities**

A number of NINDS intramural clinical studies begun in FY 2004 are investigating treatments for rare diseases. Intramural researchers are conducting a series of trials on the dosage and effectiveness of Replagal enzyme replacement therapy for Fabry disease. An NINDS pilot study has been initiated to evaluate the effect of direct current electrical polarization of the brain on patients with Pick's disease. In addition, the drug Rituximab, which has shown promise in treating other antibody-mediated disorders, is being tested for safety, tolerability, and efficacy in patients with stiff person syndrome (SPS). NINDS is also supporting clinical trials through its extramural

program for treatment of rare diseases such as amyotrophic lateral sclerosis (ALS), Canavan disease, spinal muscular atrophy (SMA), and Tourette syndrome.

NINDS, together with NIAMS and NICHD, has been actively implementing the provisions of the Muscular Dystrophy Community Assistance, Research, and Education Amendments of 2001 (the "MD-CARE Act"). In 2002, the NIH issued a Request for Applications (RFA) to establish Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers and awarded three grants in October 2003. The NINDS funds a Center at the University of Rochester that focuses on the myotonic and facioscapulohumeral forms of muscular dystrophy. In FY 2004, the NIH reissued the RFA and expects to fund up to three additional meritorious centers. Also in compliance with the MD-CARE Act, the Muscular Dystrophy Coordinating Committee (MDCC), composed of representatives from government agencies with an interest in muscular dystrophy (including NINDS, NIAMS, and NICHD) developed a Muscular Dystrophy Research and Education Plan for NIH, which was submitted to Congress in August 2004. The MDCC met in December 2004 to involve public and private sector partners in the implementation of the plan. These efforts should enhance cooperation among government and patient advocacy groups to utilize the strengths each brings to research and education in muscular dystrophy.

The SMA project is a unique program to develop therapies for SMA. The performance-based contract mechanism accelerates all steps from recognition of a research need, through solicitation, review, and funding of targeted research subprojects. An expert steering committee, with members from academia, industry, the FDA, and the NIH, actively drives the process. In its first year, the SMA Project has moved quickly. The steering committee developed detailed plans for SMA drug development, and planning for gene therapy is under way. The SMA project issued six solicitations for targeted research subprojects, and research has begun. With the ongoing consultation of the steering committee, the contractor will solicit and coordinate individual research projects in areas such as drug development, gene therapy, and stem cell therapy. This contract-based SMA translational program is the first of its kind at NINDS and may eventually serve as a model for other diseases. As a complement to the SMA Project, a workshop in September 2004 engaged the SMA scientific community, clinicians, and voluntary health organizations on development of clinical trials.

In an effort to facilitate the timely translation of promising therapies into clinical trials, NINDS established a pilot clinical trial infrastructure network called the NINDS Pilot Therapeutics Network Clinical Operations Center (NPTUNE COC). A contract has been awarded and a steering committee formed to select candidate therapies for the first pilot trials. Since the NPTUNE project will focus on disease areas with promising interventions that currently lack sufficient infrastructure and/or experience in clinical trials, many of the disease areas under consideration are rare diseases. The first pilot trial under the NPTUNE program will be for SMA. Eventually, the NINDS hopes to run two or more concurrent pilot clinical trials through the network.

**Scientific Conferences, Workshops, Symposia, and Meetings**

In FY 2004, the NINDS led or participated in the following workshops relevant to rare diseases. In most cases, the Institute collaborated with the NIH ORD or other NIH Institutes and often with patient voluntary groups.

- Annual Symposium WORLD Lysosomal Diseases Clinical Research Network
- Calcium and Cell Function
- Clinical Trials in Spinal Muscular Atrophy
- Developing Therapies for the Neurofibromatoses
- First International Conference on Ideomotor Apraxia
- Fourth International Scientific and Clinical Symposium on Tourette Syndrome
- Frontotemporal Dementia and Pick's Disease Satellite Meeting at 9$^{th}$ World Alzheimer's Congress
- Glutamic Acid Decarboxylase Autoimmunity in Batten Disease/Juvenile Neuronal Ceroid Lipofuscinosis (JNCL)
- Hershey Conference on Developmental Brain Injury
- Overcoming Neurofibromatosis Clinical Research Barriers
- Pathogenesis of Rare Neuroimmunologic Disorders
- Primary Lateral Sclerosis (PLS) Diagnostic Criteria Conference
- The Glycoproteinoses: An International Workshop on Advances in Pathogenesis and Therapy

For FY 2005, the NINDS is working with the NIH ORD on several meetings including workshops focused on drug screening for Ataxia-telangiectasia the development of clinical trials for pediatric stroke, and the brain uptake of fatty acids and lipids that relates to lipid storage diseases.

## NATIONAL INSTITUTE OF NURSING RESEARCH (NINR)

### Overview of Rare Diseases Research Activities

NINR supports clinical and basic research to establish a scientific basis for the care of individuals across the life span: from management of patients during illness and recovery to the reduction of risks for disease and disability, the promotion of healthy lifestyles, promoting quality of life in those with chronic illness, and care for individuals at the end of life. NINR's rare diseases research investigators, using an interdisciplinary approach, examine strategies to control, manage, and prevent complications.

### Recent Scientific Advances

*Childhood Acute Lymphoblastic Leukemia*

NINR-funded researchers are exploring mechanisms related to oxidative stress and chemotherapy-induced central nervous system insults. In a group of low-, medium-, and high-risk children diagnosed with acute lymphoblastic leukemia (ALL) (N = 7 in each group), support was found for the hypothesis that children in the high-risk group, observed during the most intensive ALL treatment phase, would exhibit the highest levels of oxidized phosphatidylcholine in the CNS cellular membrane (measured via cerebrospinal fluid). These findings, while only preliminary, strongly suggest a chemotherapy-induced oxidative stress association in the central nervous system and warrant further investigation.

NINR-funded investigators are also continuing to investigate math skills in ALL survivors. While previous studies have reported findings linking difficulties in math performance to children who have survived ALL, these studies have been limited by reliance on a single measure of math performance. Using multiple measures, NINR researchers reported that healthy children showed less difficulty overall in math performance compared to children who were acute ALL survivors (N = 15 in each group). Specifically, math performance in the ALL survivors was generally associated with memory function and speed in psycho-motor skills with dominant hand, while associations in healthy children were generally with basic reading and visual-motor integration skills. The researchers reported that these findings have implications for informing interventions since they elucidate the specific nature of math difficulties in ALL survivors.

*Epilepsy*

NINR researchers assessed 163 epileptic children in an investigation of the relationship between neuropsychological functioning and academic achievement. In addition, the researchers also explored the relative contributions of demographic, seizure, and psychosocial variables to the aforementioned relationship. The results revealed that children with neuropsychological deficits, exposed to a nonoptimal home environment (disorganized and nonsupportive), are at the highest risk for poor academic outcomes.

*Fibromyalgia Syndrome*

NINR-funded researchers pilot tested the feasibility of adapting an intervention, originally developed for women with multiple sclerosis, for women with fibromyalgia syndrome (FMS). The researchers postulated that although two distinct diseases, FMS and MS share commonalities that increase the probability that an intervention efficacious for women with MS could be adapted for women with FMS. This wellness intervention employs a holistic approach and focuses on empowering women by providing access to knowledge and resources that impact health-promoting or life-style behavior change. Although more work is ongoing with a larger sample of women with FMS (N = 8 in current pilot study) to test the effectiveness of the intervention, the researchers have concluded that it is feasible to adapt the intervention developed for MS to individuals with FMS.

*Irritable Bowel Syndrome*

NINR researchers recruited 144 women with irritable bowel syndrome (IBS) for a three-arm randomized self-management intervention (eight-session multi-component; single session and usual care) trial. Results revealed that a multi-component self-management program was efficacious in reducing women's gastrointestinal symptoms and improving their quality of life. Most significant is the finding that these improvements persisted up to a year post intervention.

**Rare Diseases Research Initiatives**

**Program Activities (Completed)**

End of Life: Although the field of palliative care has developed a substantial body of knowledge that addresses the needs of such patients and their families, there continues to be a dearth of persons with expertise in end of life and palliative care who are able to organize and conduct biomedical, clinical, and behavioral research in this field. A working group meeting (funded by the Office of Rare Disease) entitled ***Developing the Capacity for End of Life and Palliative Research*** was held on August 2–3, 2004, in Bethesda. The working group concluded the following: (1) there are some basic needs for end of life and palliative care research; the field needs individuals skilled at measurement and trained specifically in palliative care research design; (2) there is also a need for instruments appropriate to specific populations (e.g., children, cultural groups) and settings; (3) theoretical and conceptual frameworks are also needed to strengthen the research base. While some models exist, there was consensus among participants that more work is needed on conceptual models; and, (4) it was also concluded that there is currently much difficulty in standardizing interventions in addition to difficulties in defining outcomes. Finally, in keeping with the NIH Roadmap emphasis on multidisciplinary and interdisciplinary research, the group concluded that it is important to develop a common language across disciplines.

Biobehavioral Research: A 2-day working group meeting (funded by the Office of Rare Disease) with behavioral, biological, and immunological science experts was convened on July 15–16, 2004, in Bethesda. The workgroup titled ***Increasing Opportunities in Biobehavioral Research Utilizing Allergic Bronchopulmonary Aspergillosis (ABPA) as a Framework*** was convened to examine current knowledge and provide recommendations to further advance biobehavioral

research. Discussions focused on areas for future science directions and on the need for educating researchers in biobehavioral methods of measurement and analysis. The use of interdisciplinary/biobehavioral approaches was emphasized. Specific recommendations included the following: (1) More communication is needed between scientists in biologic and behavioral fields. This working group is one step in fostering communication, sharing information, and addressing complex issues between disciplines. (2) Patients with complex diseases have both behavioral and biologic problems. New science initiatives should encourage investigator consideration of the interaction of psychosocial, behavioral, cultural, and biological issues. (3) Biobehavioral models and frameworks should be used to guide research related to biobehavioral problems. (4) The research skills of varied scientists to do biobehavioral research is limited. Many ideas on enhancing these skills were addressed. (5) A number of biobehavioral measures were discussed and the group recommended methodological studies to improve the robustness of findings.

Non-dementing Disorders: The prevalence of non-dementing brain disorders (ND-BD, e.g., multiple sclerosis, epilepsy, Parkinson's disease, cerebral palsy) is undefined; however it is thought that as much as 5 percent of the American population may live with these "most common" brain diseases. In assessing the burden of these chronic primary diseases, little or no research has addressed the secondary effects, decreased cognitive and affective function, on physical health and quality of life. Even less is known of effective cognitive-behavioral and psychosocial interventions that may improve the health maintenance and QOL in this understudied population. A 1-day workshop (funded by Office of Rare Disease) entitled ***Promoting Research on Focal Cognitive Deficits in Non-dementing Disorder*** was convened October 12, 2004, in Bethesda, Maryland. The workgroup examined the cognitive and affective changes observed in some persons with ND-BD and identified intervention strategies to improve health behaviors, including health decision-making and QOL in these patients.

**Rare Diseases Research Initiatives**

**Program Activities (New)**

*Pediatric and Adolescent AIDS in United States*

Due to screening of the blood supply in the United States and treatment to prevent vertical transmission, HIV infection among young children in the United States is now extremely rare. However, HIV/AIDS among adolescents and young adults (13–24), while still rare among the population as a whole, is of great concern. CDC data point to several disturbing trends:[12] There were 372 new AIDS diagnoses among adolescents 13 to 19 in 2001, and 1,461 among 20 to 24 year olds that year. This likely means that the youngsters were first infected at earlier ages. AIDS prevalence has been slowly rising in these age groups since 1998. Furthermore, minority youngsters are disproportionately involved in the epidemic. Issues related to primary prevention among young people are paramount. Also, issues related to maintaining treatment adherence among those who were infected early in life, in the face of the risk taking behaviors associated with adolescence, are critical areas for further research.

[12] Futterman, DC. HIV and AIDS in adolescents. Adolescent Medicine. 15(2004): 370

A 2-day workgroup is being proposed for the summer of 2005. It is the premise of the proposed workshop that such research must be conducted in a manner that is culturally appropriate in order to develop meaningful results. Experts in HIV/AIDS research and cultural experts from nursing science, anthropology, sociology, psychology, and health communications will be invited to develop the paradigm of cultural competence as it relates to HIV/AIDS prevention, symptom management, and treatment adherence. The workgroup will be organized around general concepts of cultural competence in health promotion and health-related quality of life, with a focus on HIV/AIDS issues among younger Americans.

# NATIONAL CENTER FOR COMPLEMENTARY AND ALTERNATIVE MEDICINE (NCCAM)

## Overview of NCCAM Rare Disease Research Activities

Congress endowed NCCAM with a broad statutory mandate to conduct and support complementary and alternative medicine (CAM) research, support research training, disseminate information, and facilitate integration of CAM and conventional healthcare delivery to move the CAM field forward. To fulfill its mandate, NCCAM is undertaking a number of challenges and supporting a broad portfolio of research to expand basic and clinical research, including the prevention, evaluation, and treatment of many rare diseases. The research projects supported by NCCAM also test hypotheses for which minimal preliminary data or lack of a conventional biological rationale exists, and elucidate mechanisms of action underlying CAM practices.

## Recent Scientific Advance in Rare Disease Research

## Cancers

*Ovarian Cancer*

Investigators will address the benefits of acupuncture as adjunct therapy in the treatment of two women's health conditions. They will apply acupuncture to ovarian cancer patients with chemotherapy-induced neutropenia, and to adolescent and young women with endometriosis who experience chronic pelvic pain.

*Non-Hodgkin's Lymphoma*

Previous research has indicated that guided imagery and music therapy show promise as intervention techniques to improve mood in patients with cancer. A study on music imagery integration with standard cancer care explores the effect of music imagery therapy on patients receiving intense chemotherapy for acute leukemia or high-grade non-Hodgkin's lymphoma. Outcomes will include measures of anxiety, depression, and fatigue.

*Liver Neoplasms*

S-adenosylmethionine (SAMe) is a naturally occurring compound that is also available in supplement form. Previous research in animal model studies has shown that SAMe is anti-apoptotic in normal hepatocytes and pro-apoptotic in hepatoma cells, and that a fall in hepatic SAMe levels may eventually lead to liver injury and liver cancer. Scientists presently are using additional animal model studies to enhance the understanding of the role of SAMe in liver biology and pathology and its role in serving as a therapeutic agent. Steps towards this goal include examining SAMe's effect on hepatocyte cell cycle progression in normal and cancerous hepatocytes; elucidating SAMe's effects on apoptosis in normal versus cancerous liver cells; investigating how SAMe deficiency leads to oxidative stress; and examining whether chronic hepatic SAMe deficiency predisposes to liver fibrosis.

*Glioblastoma*

Researchers are performing a double-blind randomized controlled clinical trial in order to investigate whether distance healing (a mental intention on behalf of one person, to benefit another at a distance) using Qi Gong may have an effect on the survival time and loss of function of patients with the most common form of brain cancer, glioblastoma. Qi Gong is an ancient Chinese Taoist health exercise for rejuvenating the body's "internal energy systems." The Qi Gong sessions, conducted as a complement to standard radiation therapy, are performed at a distance, so that patients and healers never meet and patients are unaware if they are in the healing group.

*Sarcoma*

Researchers have initiated a randomized, controlled study to determine whether electroacupuncture is effective in the treating chemotherapy-induced delayed nausea and vomiting in patients with pediatric-type sarcomas, such as Ewing's sarcoma, resulting in improved management of these symptoms and enhanced quality of life. They also are studying whether acupuncture reduces the psychological stress associated with chemotherapy treatment and thus reverses the negative effects of stress on the neuroendocrine and immune systems.

**Neurological Disorders**

*Amyotrophic Lateral Sclerosis*

Scientists are using cell culture studies and relevant animal models to investigate the molecular and cellular mechanisms of action of antioxidants such as alpha-lipoic acid, ethylenediamine tetra-acetic acid (EDTA), desferrioxamine, and uric acid, and their safety and efficacy in treating amyotrophic lateral sclerosis (ALS) and other disorders. These studies will provide essential knowledge about dose-response effects, methodologies for advancing CAM therapies to human trials, and side effects. In a separate study, researchers are evaluating the nutritional supplement creatine in a Phase III clinical trial to determine if it may safely improve arm muscle strength and slow the deterioration of motor and pulmonary function of patients with ALS.

*Huntington's disease*

Creatine is a widely used dietary supplement principally taken to enhance athletic performance. Scientists are investigating whether creatine can effectively act as a neuroprotectant by preventing oxidative stress, which is conventionally associated with neuronal death in Huntingtons's disease (HD). Previous research has shown that creatine can improve behavioral and neuropathological phenotypes in HD animal models. Additional preliminary studies have shown that creatine can reduce metabolic stress in humans with HD. The investigators have since initiated a study to determine the potential mechanisms of creatine neuroprotection, test its safety and tolerability in HD patients, and examine how it impacts HD symptoms (e.g., weakness and muscle mass loss) and its progression.

**Other Rare Diseases**

*Sickle Cell Anemia*

Investigators are evaluating in-home massage therapy and in-home relaxation training to alleviate pain in African American adolescents and adults with sickle cell disease. The randomized clinical trial will measure pain, physical functioning, depression, anxiety, and health care utilization, such as physician and emergency department visits, hospitalizations, and medicine use.

*Crohn's Diseases*

Crohn's disease and ulcerative colitis (or inflammatory bowel disease, IBD), are chronic inflammatory diseases of the gastrointestinal tract. Despite current medical intervention, consisting of anti-inflammatory and immunosuppressive agents, symptoms for many patients remain untreatable. Some components of green tea, polyphenols and epigallocatechin-3-gallate (EGCG) have potent anti-inflammatory properties. The investigators proposed this study to provide the basis for determining whether and under what conditions a clinical trial of green tea polyphenols/EGCG should be conducted.

*Cystic Fibrosis*

Cystic Fibrosis (CF) is the most common genetic cause of early death in Caucasians. Preclinical evidence suggests that CF lung disease is characterized by excessive inflammatory responses. The investigators are testing their hypothesis that feverfew extracts will check the excessive inflammatory responses in mouse models of CF lung disease, by determining the effects of feverfew extracts on lung and body-wide inflammatory responses in mice with CF.

In another study, researchers are evaluating the use of probiotics in children with CF to help prevent antimicrobial resistance in these patients. *Staphylococcus aureus*, an organism frequently resistant to multiple antibiotics, is an important early cause of complications in children with CF, and is usually followed by infection with *Pseudomonas aeruginosa*. The investigators are conducting a pilot study to evaluate whether orally administered *Lactobacillus GG*, a probiotic, is effective in getting rid of *S aureus* in children with CF, and preventing them from also becoming infected with *P. aeruginosa*.

*Mucolipidosis*

The therapeutic strategies for lysosomal diseases such as mucolipidosis type IV (MLIV), Tay-Sachs, and Niemann-Pick are very limited. These devastating diseases affect mainly children and adolescents. The investigators are studying the effects of dietary agents on lysosomes in mucolipidosis.

## NATIONAL CENTER FOR RESEARCH RESOURCES (NCRR)

## Overview of Rare Diseases Research Activities

The National Center for Research Resources (NCRR) develops and supports critical research technologies and resources that facilitate biomedical research to improve the health of citizens of the United States. NCRR supports shared resources, sophisticated instrumentation and technology, animal models for the study of human disease, clinical research resources, and research capacity building for underrepresented groups.

Through its support of multidisciplinary research resources, NCRR is uniquely positioned to provide resource support in partnership with other Institutes or Centers to address emerging clinical and basic research needs, such as for the study of rare diseases. Expansion of NCRR's efforts in new biotechnologies and instrumentation, development of animal models, and clinical research resources will foster interdisciplinary collaborations and advance NIH's efforts to study rare diseases.

### Science Advances

**Ataxia-telangiectasia** (AT) is a rare, inherited disorder associated with the inability to coordinate muscle movements, nystagmus, slurred speech, involuntary muscle jerks, decreased reflexes, and, occasionally, sensory defects. In addition, patients with AT have immune deficiencies leading to recurrent pulmonary infections, leukemia, and cancer. They also have an increased incidence of diabetes. Disease severity is highly variable, with some patients wheelchair bound and dying in childhood and others living to middle age. The AT gene encodes a protein with similarities to proteins involved in cellular signal transduction and control of the cell cycle. Through the NCRR Institutional Development Award (IDeA) Program researchers at the University of South Dakota have shown that the AT gene may be responsible for several errors in the biochemical signaling pathways that regulate not only blood sugar levels but also cell growth and cell division. Understanding the failures in signaling associated with the AT gene may lead to new approaches to treating this disease and offer new insights into the fundamental biochemistry of cell growth and cell division needed to understand a number of other diseases such as cancer and diabetes.

**Chromosome 18q deletion syndrome** is a genetic disorder caused by deletion of the terminal part of the long arm of chromosome 18, resulting in multiple congenital malformations. The phenotype is highly variable but is characterized by mental retardation, short stature, hypotonia, hearing impairment, and foot deformities. Deletion syndromes of chromosome 18p- and 18q- are rare; the estimated frequency is about 1/40,000 births (approximately 100 U.S. babies per year). Researchers at the Oklahoma Medical Research Foundation in Oklahoma City are exploiting the advances made in sequencing the human and mouse genome, genetic engineering, and DNA manipulation to model the human 18q- syndrome in the mouse. Study of this mouse model should greatly accelerate insight into the pathogenesis of this syndrome at the molecular level. Knowledge gained from the model will lead to novel strategies for diagnosis and treatment of this syndrome.

**Lysosomal storage disorders** are a group of more than 30 inherited disorders resulting from the body's inability to produce certain enzymes necessary to break down and recycle large molecules. The lack of enzyme activity results in the accumulation (storage) of large molecules, which results in cell damage that can be apparent at birth or develops with aging. The diseases can affect multiple organ systems, often including the brain, resulting in mental retardation and a short life span. In the United States, these disorders are estimated to affect 1 in 7,000 births. An NCRR-supported research team at the University of Pennsylvania School of Veterinary Medicine has discovered cats and dogs with several of these diseases, including mucopolysaccharidosis (MPS) types I, IIIB, VI, and VII; alpha-mannosidosis; Krabbe disease; and mucolipidosis II. These animals have assisted in the ongoing evaluation of therapies, including enzyme replacement, bone marrow transplantation, and gene therapy using retrovirus and adeno-associated virus vectors. Gene therapy studies have been successful in preventing many of the clinical manifestations of lysosomal storage disease in these animals. These studies in large animal models are promising for the future use of gene therapy in clinical trials in humans.

**Periventricular leukomalacia (PVL**) is the principal form of perinatal brain injury and leads to cognitive and motor performance defects. The cause and mechanism of defects in PVL are not well understood. The NCRR-supported Neuroimage Analysis Center at Brigham and Women's Hospital in Boston applied its imaging tools to the study of PVL. This study used magnetic resonance imaging (MRI) and the Center's analysis tools to quantitatively assess the damage associated with this disease in different structures in the brain, including the cortex, basal ganglia, cerebellum, and white matter fiber tracts. The study has provided insight into potential causative factors of this poorly understood disease. It has also established an important quantitative baseline against which future treatments of the disease can be assessed.

**Retinal degeneration** is seen in several rare diseases associated with neural degeneration. Under the auspices of an NCRR-supported K23 career development award, patterns of gene expression and specific protein synthesis in normal mice and in a mutant mouse strain that exhibits retinal degeneration as a result of apoptosis of photoreceptors were compared. The researchers identified a panel of signaling and apoptosis-associated molecules previously not known to be involved in photoreceptor degeneration. Several of these proteins occupy central positions in known signaling pathways not previously associated with retinal degeneration.

In addition, using a mouse model system, NCRR-supported researchers at the University of Oklahoma Health Sciences Center are investigating the molecular and cellular basis for the observed loss of retinal photoreceptor cells when two defective copies of a particular photoreceptor gene are inherited. Recently, they showed that direct injections of certain growth factors delay retinal degeneration in these mice by turning on conserved cellular signaling pathways that promote cell survival. Further studies to understand the mechanism of protection may lead to new therapies that prevent or slow the onset of blindness in affected persons.

## Significant Ongoing Rare Diseases Research Initiatives

### Rare Diseases Clinical Research Network

The Rare Diseases Clinical Research Network (RDCRN), a collaboration between NCRR and the NIH Office of Rare Diseases with support from several other NIH Institutes, has grown with the addition of three new Clinical Research Consortia. Thus, the RDCRN currently consists of 10 Rare Diseases Clinical Research Consortia (RDCRC) and the Data and Technology Coordinating Center (DTCC). This past year, while each RDCRC focused on development of clinical protocols for a subset of related rare diseases, the DTCC developed and enabled technology, tools, and services for the network, including electronic data entry, remote direct laboratory transfer, vocabulary and laboratory standards, statistical support, Web site development and maintenance, and database querying tools. The RDCRN vocabulary unit has been instrumental in incorporating standards across networks and development of an adverse event reporting system. In addition, in collaboration with national standards organizations, including the Systematized Nomenclature of Medicine Clinical Terms (SNOMED CT), Health Level Seven, and the National Library of Medicine, the DTCC has facilitated the incorporation of unique concepts and terms necessary for rare diseases in national vocabularies.

The patient support groups affiliated with each of the consortia have formed the RDCRN Coalition of Patient Advocacy Groups (CPAG) to work together to support each other in their outreach efforts to patients afflicted with rare diseases, their families, and the public. One of their first projects is to develop information on the diseases targeted in the RDCRN for emergency room physicians, who may be seeing an individual with a rare disease for the first time. The DTCC, in collaboration with each RDCRC, has implemented a patient contact registry, which allows individuals with each of the targeted rare diseases to register to receive information about new or ongoing clinical studies or trials in the Consortia in addition to periodic educational updates from the network. While no studies began in the first year, many Consortia have been busy with the development of longitudinal studies as well as clinical trials of potential new therapeutic agents. Most Consortia have begun training programs focused on the creation of a cadre of clinical investigators interested in rare diseases. The RDCRN Web site (http://www.rarediseasesnetwork.org) is a source of information for the public, physicians, patients, and investigators about rare diseases. The patient contact registry is Web enabled and accessible from the Web site.

### Industry-initiated Rare Disease Research Supported at General Clinical Research Centers (GCRCs)

The NCRR-supported GCRCs host much of the research focused on rare diseases, as there are few large clinics available specifically for these unusual disorders. While most of these studies are investigator initiated, there are industry-sponsored phase 1, 2, and 3 trials of new interventions that utilize the GCRCs. Since the number of affected individuals is small, the expected market is also small, limiting the interests of many pharmaceutical companies. Recognizing the need to advance the development of new agents for these diseases, the Orphan Drug Act provides incentives for companies to develop and license agents for rare diseases. The NCRR, also recognizing the need

and benefit from support of new agents for rare disease, modified the guidelines for GCRCs for industry-initiated studies and trials. Normally, companies that wish to utilize the resources of the GCRCs in the performance of their studies must pay for those resources. For industry-supported rare disease studies and trials, the GCRC may approve use of GCRC resources without charge. Several rare disease studies are already benefiting from this change, including cystic fibrosis, lysosomal storage diseases, and urea cycle disorders.

**Activities with Nonprofit Organizations**

In partnership with the Cystic Fibrosis Foundation (CFF), NCRR supports a novel approach to develop new therapeutics for cystic fibrosis, a rare genetic disease. The CFF Therapeutics Development Network (TDN) unites investigators focused on cystic fibrosis research to perform clinical trials of promising agents for treatment and cure of this disorder. NCRR-supported GCRCs, which provide personnel, resources, and space for the conduct of clinical research, are utilized by many investigators in this network. In addition, NCRR supports a coordinating center, which provides informatics support for the management, conduct, and analysis of the studies. This bioinformatics component includes a secure, interactive Web environment for network communication and data entry as well as a biostatistical unit. These resources facilitate transfer of new discoveries from bench to bedside.

The National Disease Research Interchange (NDRI) is a not-for-profit organization in Philadelphia, founded in 1980. NCRR provides support for approximately one-third of their activities via a cooperative agreement. NDRI personnel obtain commitments from academic pathologists to provide human tissues for basic research and statements of need according to specific protocols from biomedical researchers. These two lists, with very specific clinical details (but without patient identifiers), are kept in NDRI databases. When a tissue becomes available, a researcher is contacted by NDRI staff and asked if he/she can accept it. Upon positive reply, the pathologist is notified; prepares the tissue according to the researcher's protocol; and sends it, anonymized, to the researcher. The researcher pays a relatively small fee. Through this cooperative agreement, NDRI facilitates laboratory research on a broad variety of rare and common diseases. The agreement is currently co-funded by NCRR, the Office of Rare Diseases, and four other NIH Institutes and Centers.

**Rare Disease-specific Conferences, Symposia, or Meetings**

In collaboration with the Office of Rare Diseases, NIH, NCRR organized a meeting of the representatives of the patient advocacy groups that are associated with the Rare Diseases Clinical Research Network on July 28 – 29, 2004. This meeting provided an opportunity for all of the lay groups working with the Rare Diseases Clinical Research Consortia to share experiences and explore ways that their efforts could be synergized. The morning session provided an overview of how NIH works, the "Common Rule" from the Office of Human Research Protections, and the Health Insurance Portability and Accountability Act (HIPAA) from the NIH Office of the Director. The afternoon was composed of four panels, each with three representatives from member advocacy groups. These sessions discussed Recruitment, Web Resources, Dissemination of Study Results to Patient and Physician Communities, and Mechanisms for Working Together across Diseases. As a result of this meeting, the RDCRN CPAG was formed. All present were very

excited about the sharing that went on at the meeting and working together on shared goals. Co-leaders of the CPAG were elected and two subcommittees were organized. One subcommittee will work to develop, in collaboration with their Consortia, information for emergency room physicians who may be encountering a patient with a rare disease for the first time. The other subcommittee will work to develop best practices, resources, and practical guidelines for obtaining Continuing Medical Education credits for physicians and nurses for rare disease meetings. The two large umbrella organizations for rare diseases, NORD and the Genetic Alliance, actively participated and are part of the new CPAG.

## NATIONAL LIBRARY OF MEDICINE (NLM)

### Overview of Rare Diseases Research Activities

The National Library of Medicine (NLM) provides information resources useful to rare disease research and to those seeking information about conditions that affect them or their families.

### Database Resources

- Citations to articles on rare diseases have long been available in the MEDLINE database, now accessible to researchers, health professionals, and the public through NLM's free Web-based PubMed system, and also in the TOXNET system. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi and http://toxnet.nlm.nih.gov/.
- MedlinePlus, NLM's consumer health information service, has a general rare diseases page that has been effective in referring members of the public to the NIH Office of Rare Diseases at http://www.nlm.nih.gov/medlineplus/rarediseases.html and was accessed 15,655 times during FY 2004. MedlinePlus also incorporates links on health topic pages to Genetics Home Reference, a new NLM database that includes many rare diseases. Currently there are 125 links from MedlinePlus to Genetics Home Reference topics such as amyotrophic lateral sclerosis, Gaucher disease, and Marfan syndrome. In addition, MedlinePlus has continued to add topics on specific rare diseases requested by consumers; examples during FY 2004 included pulmonary hypertension, ataxia telangiectasia, anal cancer, premature ovarian failure, and rickets.
- Online Multiple Congenital Anomaly/Mental Retardation (MCA/MR) Syndromes, http://www.nlm.nih.gov/mesh/jablonski/syndrome_title.html, a database of structured descriptions of congenital abnormalities (many of them rare) associated with mental retardation. This database was searched nearly ½ million times in 2003.
- The Genetics Home Reference (GHR) is the NLM's Web site for consumer information about genetic conditions and the genes and chromosomes responsible for those conditions. GHR's integrated Web-based approach provides brief, consumer-friendly summaries of genetic conditions and related genes and chromosomes. Understanding is enhanced by direct links to glossary definitions and a handbook called *Help Me Understand Genetics* that explains the fundamental genetic concepts. Additional links to consumer information from MedlinePlus, applicable clinical trials, and relevant patient support groups are provided. Each summary also includes links to advanced information from the NLM and other authoritative sources. GHR currently offers summaries on more than 119 genetic conditions, including numerous rare diseases and disorders, 186 genes, and each of the 23 pairs of human chromosomes. New content is added and updated on a regular basis and reviewed by experts in human genetics. http://ghr.nlm.nih.gov/
- ClinicalTrials.gov, NLM's consumer health information system for linking patients to medical research, currently includes approximately 12,200 studies. Of these, about 5,714 represent approximately 850 rare disease conditions. Also, since FY 2003, study records in ClinicalTrials.gov, including those investigating rare diseases, were linked to relevant genetic condition summaries in GHR. Such links provide consumers seeking information about clinical trials with additional background about the conditions and the genes responsible for the conditions. http://clinicaltrials.gov/ and http://ghr.nlm.nih.gov/.

**Research Support**

- NLM, working in partnership with organizations in Africa, the United States, the United Kingdom, and Europe, has created MIMCom.Net, the first electronic malaria research network in the world. Using satellite technology, the network provides full access to the Internet and the resources of the World Wide Web as well as access to current medical literature for scientists working in Africa. The African research sites are of recognized high quality, require improved communications to accomplish ongoing research, and have the necessary resources to purchase equipment and sustain the system. http://www.mimcom.net/
- NLM is assisting NCRR in establishing the NIH-funded Rare Disease Clinical Research Network as an early test-bed for the use of standard clinical vocabularies to improve the efficiency of clinical research.
- A Multicenter Clinical Trial Using Next Generation Internet (NGI) Technology. NGI technology was applied to provide the infrastructure of a multicenter clinical trial of new therapies for adrenoleukodystrophy (ALD), a fatal neurologic genetic disorder. This project involved the formation of a worldwide imaging network of clinical institutions to evaluate ALD therapies. This network was required to provide a sufficient number of patients for evaluating ALD therapies. This can serve as a model for many other disorders. Three centers collaborated on this project: the Imaging Science and Information Systems (ISIS) Center at Georgetown University Medical Center, the Kennedy Krieger Institute, and the Department of Radiology at Johns Hopkins University. NGI technology was used to speed the transmission and evaluation of high-quality MRI images. Another important feature of this project was to gain insight into procedures that ensure medical data privacy and security. http://www.nlm.nih.gov/research/ngisumphase2.html

**Grants**

- Kawasaki disease (KD) is an acute, self-limited illness of infancy and early childhood that has now replaced rheumatic fever as the leading cause of acquired heart disease in children in the United States and Japan. Although the acute illness resolves spontaneously, permanent damage to the coronary arteries occurs in 20–25 percent of untreated children. The cause of KD remains unknown and there is no specific laboratory test to identify affected children. Nonetheless, an effective treatment exists that significantly reduces the risk of coronary artery damage. KD thus presents a unique dilemma: the disease may be difficult to recognize, there is no diagnostic laboratory test, there is an extremely effective therapy, and there is a 25-percent chance of serious cardiovascular damage or death if the therapy is not administered. NLM is funding a publications grant to the Kawasaki Disease Foundation to support the continued collaboration of an unusual multidisciplinary team with expertise in documentary film making, parent advocacy, pediatric medicine, anthropology, and the history of medicine to produce a Web-based archive of interviews and a television documentary to increase public awareness of KD and to support scholarly research on the origins of this emerging pediatric disease. Funds from this application will support three major interviewing sessions in Japan, Hawaii, and San Diego. The film will focus on (1) the importance of informed parents in establishing the timely diagnosis of KD, which permits effective treatment and prevention of complications and (2) the history of KD, showing that the ways in which it emerged as an internationally recognized disease mirror the ways in which it is now diagnosed or misdiagnosed in our contemporary health care system. In the case of KD, informed parent advocacy can mean the difference between life and death for an affected child.

- Hepatitis B is the world's most common serious liver infection and is associated with more than 80 percent of liver cancer worldwide. Those that are affected are in urgent need of information to help them to successfully deal with their disease and maintain a high quality of life. The long-range goal of this information system grant to the Hepatitis Foundation is to provide information and support to the millions of individuals worldwide that are affected by hepatitis B. In particular, this project is developing a comprehensive hepatitis B Web site to bring high-quality health information to consumer users and health care professionals. The creation of this "virtual community" includes high-technology features such as an e-newsletter and expert forums, delivered in a user-friendly package. Additionally, Web site content and features will be personalized in response to the specific needs and preferences of the target audiences. This project also focuses on creating an important connection between information seekers and the National Library of Medicine databases, including MEDLINE, MEDLINEplus, and ClinicalTrials.gov. The Web site will be evaluated throughout the 3-year timeline to incorporate user feedback into the development process, to track Web site usage statistics, and to determine the impact of the Web site on the targeted users. Ultimately, the completed Web site can be utilized as a model for the interactive dissemination of high-quality health information through the Internet.

**Rare Diseases Research Initiatives**

The National Center for Biotechnology Information (NCBI), a division of NLM, serves as a national public resource for molecular biology information. In this capacity, NCBI establishes and maintains various genomic databases and develops software tools for mining and analyzing this data, all of which are freely available to the biomedical community to facilitate a better understanding of the processes affecting human health and disease.

*The Human Genetic Map*

NCBI is responsible for collecting, managing, and analyzing the growing body of data being generated from the sequencing and mapping initiative of the Human Genome Project. At present, NCBI makes the sequence of the entire human genome, with its complement of over 28,000 known and predicted genes, available without restriction to the public. This unrestricted access has expedited the decoding by the scientific community of important human genes and, as a result, scientists are beginning to understand the causes of many rare diseases. Access to the complete human genome and the related genetic data at NCBI helps scientists determine the organization of the genes on a chromosome, study how these genes produce their protein products, investigate how changes in a gene’s DNA sequence give rise to a disease-causing mutation, and study how chromosomes are duplicated and inherited. Scientists have used these strategies to study gene defects on chromosomes 21 and 22 that lead to a variety of rare diseases, including Downs syndrome, Usher syndrome, DiGeorge syndrome, and Ewing’s sarcoma. NCBI investigators have also played an instrumental role in the identification and analysis of other disease genes and genetic loci and have analyzed genetic data leading to scientific advances in the understanding of several rare diseases and disorders, such as the identification and analysis of the genes for Kallmann syndrome and neurofibromatosis (NF1). Examples of other rare diseases currently being studied by NCBI investigators include ataxia telangiectasia, breast cancer, hyper-IgE syndrome, nemaline myopathy, and obesity.

*Genetic Analysis Software*

NCBI investigators are working to develop, implement, and disseminate high-performance computational tools and application software packages for the analysis of genetic data and its linkage to disease. Several of these software packages are described below.

FASTLINK is a computer program designed by NCBI investigators to analyze the associations between genes and genetic markers that lie near each other on a chromosome, a process called "genetic linkage analysis." Genes and other genetic markers that are linked are often inherited together and, therefore, can be used to map the location of a disease gene. NCBI investigators have used FASTLINK to study hyper-IgE syndrome, a rare immunodeficiency characterized by recurrent skin abscesses, pneumonia, and highly elevated levels of serum IgE. Using FASTLINK, researchers were able to find evidence linking this syndrome to chromosome 4. FASTLINK has been cited in over 400 other published genetic studies, including studies of macular dystrophy, type 1 hereditary sensory neuropathy, and Alstrom's syndrome.

CASPAR (Computerized Affected Sibling Pair Analyzer and Reporter) is a computer program designed by NCBI investigators to study the genetics of complex diseases, or diseases involving the interaction of multiple genes. It allows a scientist to explore various hypotheses about how different factors may be involved in disease susceptibility. NCBI investigators have used CASPAR to study linkage analysis in patients with a form of diabetes.

The PedHunter computer program was developed to query genealogical databases to uncover connections between relatives that are afflicted with the same disease and to construct a pedigree suitable for genetic linkage analysis. NCBI investigators are using PedHunter to query the Amish genealogy database to collect information on various genetic diseases, including nemaline myopathy, a rare genetic neuromuscular disorder that is usually apparent at birth and is characterized by extreme muscle weakness. Using PedHunter, in combination with other genetic analysis software, NCBI investigators have demonstrated that, in the Amish, this disorder is caused by a mutation in the gene for the sarcomeric thin-filament protein, slow skeletal muscle troponin T (TNNT1). TNNT1 maps to chromosome 19 and has been previously sequenced. Further analysis resulted in the identification of a stop codon that segregated with the disease. Researchers concluded that Amish nemaline myopathy is a distinct, heritable, myopathic disorder caused by a mutation in TNNT1.

The Comparative Genomic Hybridization (CGH) analysis software package is being used by NCBI investigators for modeling the process of tumor formation in various forms of cancer.

The function of the software is to develop models that relate genetic aberrations with tumor progression. Investigators have used CGH as part of a larger project to search for and identify possible susceptibility loci involved in both breast and bladder cancer. Investigators have also published the results of a case study in which CGH was used to analyze chromosomal abnormalities in a large collection of ovarian cancer samples.

*Three-dimensional Structure Database*

NCBI's Structure Research Group maintains a database of experimentally determined three-dimensional biomolecular structures as well as tools for visualizing and analyzing these structures. Three-dimensional structures provide a wealth of information on the biological function of a molecule, on mechanisms linked to function, and on evolutionary history of and relationships between macromolecules—all valuable clues leading to a better understanding of rare diseases.

For example, in 1995, the structure of leptin—the protein coded within a gene linked to obesity and diabetes—was predicted by NCBI investigators using the structure database. After the discovery of leptin, researchers analyzed the protein's sequence and determined that it exhibited no similarities to other known proteins. NCBI investigators hypothesized that leptin was ancestrally related to at least one other protein whose sequence had diverged such that only a comparison of three-dimensional structures might detect a relationship. Investigators conducted a search of the database to determine whether this protein might adopt a similar fold pattern, or structure, to that of a protein structure already stored in the database. They discovered that leptin's sequence was compatible with the structure of a family of known proteins and predicted a structural model based on these results. Subsequently, this early prediction was confirmed by cloning of the protein's receptor, and more recently, by x-ray structure determination. Now that the structure of leptin has been confirmed, future studies of leptin as well as other leptin-regulated genes may reveal the mechanisms by which leptin exerts its effect on the body.

*Malaria Genetics and Genomics*

Human malaria is caused by four *Plasmodium parasites*: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Although *P. vivax* is widespread, *P. falciparum* is the most severe and lethal tropical parasite, leading to an estimated 1–2 million deaths each year, mostly of children in Africa.

The resurgence of malaria in recent years is mainly attributed to the emergence and spread of multiple-drug-resistant parasites and insecticide-resistant mosquito vectors, presenting a serious problem for travelers and the military in malaria-endemic regions as well as for the resident populations. Accordingly, much research at the NIH focuses on the treatment and prevention of malaria, which is a curable disease if promptly diagnosed and adequately treated.

The NCBI, in collaboration with the NIAID, has supported the efforts to sequence and analyze the complete genome of *P. falciparum* and related parasites, thereby providing researchers with access to information relating to all of the genes found in these parasites. Analyses of these genomic data by NCBI researchers are contributing to enhanced understanding of this complex disease and attempts to develop improved anti-malarial drugs, vaccines, and other control strategies. Moreover, a collaborative team of NIH investigators, including researchers from the NCBI, have constructed a genome-wide, high-resolution genetic linkage map of *P. falciparum*. The computer analyses based on these genetic parameters and markers have facilitated genome sequence assembly and are currently helping to define the genes involved in parasite resistance to multiple drugs and to trace the evolution and spread of these genes in parasite field populations in Africa, Asia, and the Americas.

NCBI's Malaria Genetics and Genomics Web page serves as an information and data resource covering *Plasmodium* and related parasites, including rodent malarias and the *Anopheles gambiae* mosquito vector. This resource includes links to the sequence and genomic data in Entrez Genomes and other NCBI databases together with unique information on genome maps, linkage markers, and genetic studies. Links are provided to various malaria research projects being conducted at NIH, to NIAID's Malaria Research and Reference Reagent Resource Center, and to other malaria-related sites.

The NCBI, in collaboration with the WHO's Special Programme for Research and Training in Tropical Diseases (TDR) and other international partners, has continued to support the international outreach efforts to train scientists in developing countries to use current bioinformatics tools and genomic data, such as the mosquito and malaria genome, for their own research. NCBI staff have provided coordination and instruction at several international bioinformatics training courses and centers in Africa, Asia, and South America, including the WHO/TDR sponsored Regional Training Course in Bioinformatics Applied to Tropical Diseases.

*Additional Human Genome Resources*

NBCI makes available a number of other resources to facilitate the widespread use of human sequence data. The Human Genome Resources Web page serves as a focal point for biomedical researchers from around the world, enabling them to use this data in their research. From the Human Genome Resources Web page, researchers can access the NCBI Map Viewer, which presents a graphical view of the available human sequence data in conjunction with cytogenetic, genetic, and physical maps. Researchers may quickly search for a gene or a gene marker of interest by querying against the entire human genome. Query results link to a graphical display of the gene or gene marker within the context of additional data. The coupling of the human genome sequence with genetic and physical maps bearing markers associated with disease allows researchers to identify candidate genes for further research. The NCBI Map View also allows the maps and genomic sequences of organisms used in models of human disease, such as mouse and rat, to be viewed alongside the human maps. The ability to compare sets of genomes in this manner allows researchers to use the results obtained in their laboratories with these model organisms to better understand the roots of human disease.

NCBI’s Genes and Disease Web page is designed to introduce visitors to the relationship between genetic factors and human disease.

Genes and Disease provides information for more than 80 genetic diseases, including many rare diseases. The Online Mendelian Inheritance in Man database, or OMIM, is a continuously updated catalog of inherited human disorders and associated sequence mutations, authored and edited by Dr. Victor A. McKusick and colleagues and developed for the Web by NCBI. OMIM now contains over 15,000 entries for diseases linked to over 9,000 locations on the human genome.

One of the primary reasons for sequencing the human genome was to gain an understanding of the role of genes in human disease. By studying the gene sequences associated with a human or model organism disease, researchers can gain important insights into the genetic and environmental basis of disease. The advances outlined here demonstrate the importance and utility of NCBI’s computer

databases, data analysis tools, and software algorithms in identifying and understanding human disease genes and pave the way for the development of novel strategies to diagnose, treat, and ultimately, prevent disease.

*Severe Acute Respiratory Syndrome Coronavirus Resource*

The severe acute respiratory syndrome (SARS) virus was responsible for an outbreak of severe, atypical pneumonia in Guangdong Province, China, in late 2002. The disease had an extremely high mortality rate (up to 19 percent) and expanded rapidly to other countries. In April 2003, a previously unknown coronavirus was isolated from patients and subsequently shown to be the causative agent in experiments on monkeys. The first complete sequence of SARS coronavirus was produced by the BCCA Genome Sciences Centre, Canada, about 2 weeks after the virus was detected in SARS patients. It was submitted to the NCBI's GenBank sequence database, and the NCBI Viral Genomes Group annotated the sequence and released it the next day. The availability of sequence data and the functional dissection of the SARS-CoV genome at NCBI has been a necessary prerequisite for developing diagnostic tests, antiviral agents, and vaccines. The NCBI Web resource includes the complete sequence as well as links to the latest sequence data and publications and results of pre-computed sequence analyses: genomic, protein, and structural.

*Database of the Major Histocompatibility Complex*

The NCBI dbMHC database provides an open, publicly accessible platform for DNA and clinical data related to the human major histocompatibility complex (MHC). MHC research and clinical data generated at meetings such as the International HLA Workshop and Congress has proven valuable to the international research community. NCBI makes these data available along with tools for submission and analysis of research data linked to the MHC. The dbMHC contains reagent data used for tracing DNA typing and a section with anonymous clinical data from MHC-related research projects related to diseases such as celiac disease, narcolepsy, ankylosing spondylitis, and hemochromatosis.

## OFFICE OF RARE DISEASES (ORD)

### Overview

The Office of Rare Diseases (ORD) was established in 1993 within the Office of the Director of the National Institutes of Health (NIH). On November 6, 2002, the President established the Office in statute (Public Law 107-280, the Rare Diseases Act of 2002). The Act defines a rare disease as a disease or condition affecting fewer than 200,000 persons in the United States and estimates that 25 million people in the United States have a rare disease.

The goals of ORD are to stimulate and coordinate research on rare diseases and to support research to respond to the needs of patients who have one of the more than 6,000 rare diseases known today. To leverage its resources, stimulate rare diseases research activities, and foster collaboration, ORD works with NIH Institutes or Centers to support

- an extramural research program that includes a network of clinical research centers on rare diseases and the training of rare diseases researchers;
- an intramural research program for patients with rare conditions and programs to stimulate clinical research on rare diseases, including the training of researchers interested in rare diseases and in clinical and biochemical genetics and in clinical and biochemical genetics;
- a scientific conferences program in response to scientific opportunities or to stimulate research where little exists or where research progress may have stalled;
- an information center to supply reliable and valid information to the public, researchers, and health care providers, including various databases to provide access to information over the Web and a number of educational activities including regional workshops to assist national patient advocacy groups become partners with the NIH by developing better understanding of NIH research programs.

### Scientific Advances

*Extramural Research Program*

- **The Rare Diseases Clinical Research Network**

  Since FY 2003, ORD and several NIH Institutes and Centers support the Rare Diseases Clinical Research Network. In FY 2004, the NIH increased the number of research consortia from seven to ten and added support for pilot studies and demonstration projects available to each consortium. Collaborating NIH Institutes and Centers include NCRR, NHLBI, NICHD, NIAMS, NIDDK, and NINDS.

  At this time, the 33 clinical protocols are under development. The collaborating patient advocacy groups have established a coordinating coalition and are represented on the network's steering committee. The network consists of more than 70 sites and 30 patient advocacy groups and conducts research on almost 50 rare diseases.

The vast distribution of research locations across the United States will make investigational treatments more accessible to patients with rare diseases. The network systematically collects clinical information to develop biomarkers and new approaches to diagnosis, treatment, and prevention of rare diseases; to provide training of new clinical research investigators; and to support demonstration projects in the following rare disease groups:

- Urea cycle disorders including citrullinemia, argininosuccinic aciduria, hyperargininemia, and others.
- Genetic developmental disorders, including Angelman syndrome, Rett syndrome, and Prader-Willi syndrome.
- Vasculitides including Wegener's granulomatosis, Takayasu arteritis, and Churg-Strauss syndrome.
- Bone marrow failure including aplastic anemia, myelodysplastic syndromes, paroxysmal nocturnal hemoglobinuria, and large granular lymphocyte leukemia.
- Rare genetic defects in steroidogenesis leading to congenital adrenal hyperplasia, androgen receptor defects, and low renin hypertension.
- Rare lung diseases including lymphangioleiomyomatosis, alpha-1 antitrypsin deficiency, pulmonary alveolar proteinosis, and hereditary interstitial lung disease.
- Nervous system channelopathies including Andersen-Tawil syndrome (periodic paralysis), non-dystrophic myotonic disorders, and episodic ataxias.
- Genetic impairments in mucociliary clearance including primary ciliary dyskinesia, cystic fibrosis, pseudohypoaldosteronism, and other chronic sinopulmonary diseases.
- Genetic causes of intrahepatic cholestasis including rare liver diseases associated with alpha-1-antitrypsin deficiency, Alagille syndrome, progressive familial intrahepatic cholestasis, bile acid synthesis defects, and mitochondrial hepatopathies.
- Rare Thrombotic Diseases Consortium including antiphospholipid antibody syndromes, heparin-induced thrombocytopenia, paroxysmal nocturnal hemoglobinuria, catastrophic antiphospholipid antibody syndrome, and thrombotic thrombocytopenic purpura.

The three new consortia are:

**1. Rare Thrombotic Diseases Consortium**

Rare disorders that are associated with an increased thrombotic risk include the antiphospholipid antibody syndromes (APS), heparin-induced thrombocytopenia (HIT), combined thrombophilic states, paroxysmal nocturnal hemoglobinuria, thrombotic thrombocytopenic purpura, and the catastrophic "thrombotic storm." These disorders frequently exhibit more "aggressive" clinical phenotypes, affecting arterial, venous, and/or microvascular beds. Diagnostic and/or therapeutic limitations exist for each of these disorders, and prospective studies are needed to more clearly define the syndromes and develop better therapies. This multi-institutional academic consortium focuses on rare thrombotic disorders and will establish a Rare Diseases Clinical Research Consortium focusing on rare thrombotic disorders.

Investigators from four academic centers will bring together existing registries (e.g., The Antiphospholipid Syndrome Collaborative Registry) and programs [e.g., the Centers for Disease Control and Prevention (CDC)-sponsored Thrombophilia Programs and the Duke Center for Human Genetics] to identify and enroll patients into hypothesis-driven prospective clinical trials that focus on:

- Genetic analysis of familial APS, familial APS/autoimmune syndromes, and patients with catastrophic "thrombotic storms."
- Identify risk factors for thrombosis in patients with antiphospholipid antibodies and HIT.
- Define the natural history of patients with elevated heparin-platelet factor four antibodies after bypass.
- Emerging opportunities from ongoing studies will be identified that promote new research directions, projects, and translational activities that foster links between the consortium and industry.
- Develop a training program for new investigators who are interested in studying rare thrombotic disorders. A program will be instituted that combines opportunities in clinical management as well as epidemiologic, genetic, diagnostic, and therapeutic investigations involving patients with rare thrombotic disorders.
- Develop a Web site that promotes education and research activities involving patients with rare thrombotic disorders. The Web site will be developed with the Data Technology and Coordinating Center and other Rare Diseases Consortia and will be for patients, healthcare providers, and the general public.

The following sites form the consortium: Duke University Medical Center, Durham, NC; University of North Carolina at Chapel Hill, Chapel Hill, NC; University of Wisconsin, Madison, WI; Mayo Clinic, Rochester, MN; and the Centers for Disease Control and Prevention, Atlanta, GA

**2. Genetic Disorders of Mucociliary Clearance Consortium**

The project director will establish a Rare Diseases Clinical Research Center (RDCRC) at The University of North Carolina (UNC) and an associated consortium with three other geographically dispersed sites to study rare diseases of the airways. These four sites in the consortium will collaborate in diagnostic, genetic, and other studies in patients with genetic impairments in mucociliary clearance, specifically primary ciliary dyskinesia (PCD), variant forms of cystic fibrosis (CF), and pseudohypoaldosteronism (PHD). Patients with these unusual disorders with increased morbidity and mortality often have delayed (or incorrect) diagnoses, because diagnostic tests are not readily available. The central hypotheses are that a broad-based systematic approach to the diagnostic evaluation of these patients will yield more precise diagnostic criteria and better diagnostic techniques including genetic testing and that systematic evaluation of specific cohorts of these patients with state-of-the-art methodologies and rigorous cross-sectional and longitudinal study designs will provide better understanding of clinical pathogenesis of these disorders. In a 5-year longitudinal study of 300 patients with PCD, investigators will use innovative techniques, including measurement of pulmonary function tests and high resolution computed tomography of the chest in infants, to define early onset and progression of pulmonary disease in PCD. Moreover, 10 additional geographically dispersed sites will serve as PCD Clinical Centers to assist in the

follow-up care of PCD patients in the longitudinal study. This collaborative effort will improve care by defining clinical practice guidelines, especially for PCD. The pilot projects in PCD are designed to develop better diagnostic tools, biomarkers, and screening tests; to characterize the respiratory pathobiology; and to evaluate novel therapeutic agents. The existing training program in rare airway diseases will be extended to established and young investigators. The consortium will work with the Data and Technology Coordinating Center to coordinate and expand current Web sites to provide information to the lay public, patients, and medical professionals for education, referral, and recruitment of study subjects.

The following sites form the consortium: University of North Carolina at Chapel Hill, Chapel Hill, NC; Washington University in St. Louis, MO; The Children's Hospital, Denver, CO; The Children's Hospital and Regional Medical Center, Seattle, WA.

**3. Rare Liver Diseases Consortium**

The investigators proposed the development of a coordinated and integrated rare liver diseases clinical research consortium. The consortium will include sites at the Children's Hospital in Denver and clinical sites within the United States that are listed below, each with investigators who have extensive clinical experience, patient populations, and research programs for these disorders and each with a General Clinical Research Center. The consortium will focus on investigations of five genetic causes of intrahepatic cholestasis. These disorders have serious if not fatal consequences (without liver transplantation) and severely affect the children's normal growth and development. The five related disorders are α-1-antitrypsin deficiency, Alagille syndrome, progressive familial intrahepatic cholestasis (PFIC), bile acid synthesis and metabolism defects, and mitochondrial hepatopathies.

The consortium will develop a longitudinal hypothesis-driven database study of these diseases. During this study, serum, DNA, and liver tissue will be obtained from all patients and stored for future studies. The consortium will also include:

- biological core facilities to ensure the highest quality analysis of genetic information, liver histopathology, and bile acid biochemistry for subjects enrolled in this study;
- a pilot/demonstration project program to encourage innovative scientific investigation;
- a training program in order to attract new investigators to the study of rare liver diseases; and
- development of electronic internet-based clinical, educational, histologic, and research resources for these diseases.

Input by support/advocacy groups for these rare liver disorders will be integrated into the consortium at all levels. The consortium will be a full partner in the Rare Diseases Clinical Research Network and will participate collaboratively with the other clinical research consortia and the Data and Technology Coordinating Center.

The following sites form the consortium: The Children's Hospital Denver, Denver, CO; Children's Hospital of Philadelphia, Philadelphia, PA; Cincinnati Children's Hospital Medical Center, Cincinnati, OH; King's College, London, England; Mount Sinai School of Medicine, New

York, NY; University of California/San Francisco, San Francisco, CA; and the University of Colorado Health Sciences Center, Denver, CO.

Table 1 lists the 10 consortia and the data and technology coordinating center, the principal investigators, and the primary performance site:

**Table 1: The Rare Diseases Clinical Research Network**

| Consortium Title | Principal Investigator | Primary Performance Site |
|---|---|---|
| **1. Urea Cycle Disorders Consortium** | Batshaw, Mark L., M.D. | Children's National Medical Center, Children's Research Institute (CNMC), Washington, DC |
| **2. Angelman, Rett, and Prader-Willi Syndrome Consortium** | Beaudet, Arthur L., M.D. | Baylor College of Medicine, Houston, TX |
| **3. Consortium for Clinical Investigations of Neurological Channelopathies (CINCH)** | Griggs, Robert C., M.D. | University of Rochester School of Medicine, Rochester, NY |
| **4. Idiopathic Bone Marrow Failure & Cytopenia Clinical Research Consortium** | Maciejewski, Jaroslav P., M.D., Ph.D. | Cleveland Clinic Foundation, Cleveland, OH |
| **5. Vasculitis Clinical Research Consortium (VCRC)** | Merkel, Peter A., M.D., Ph.D. | Boston University Medical Center, Boston, MA |
| **6. Rare Genetic Steroid Disorders Consortium** | New, Maria I., M.D. | Weill Medical College of Cornell University at the New York Presbyterian Hospital, New York, NY |
| **7. Rare Lung Diseases Consortium** | Trapnell, Bruce C., M.D. | Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, OH |

| | | |
|---|---|---|
| **8. Rare Thrombotic Diseases Consortium** | Ortel, Thomas L, M.D., Ph.D. | Duke University Medical Center Durham, NC |
| **9. Genetic Disorders of Mucociliary Clearance Consortium** | Knowles, Michael R., M.D. | University of North Carolina at Chapel Hill, Chapel Hill, NC |
| **10. Rare Liver Diseases Consortium** | Sokol, Ronald J., M.D. | Children's Hospital, Denver, CO |
| **Rare Diseases Data and Technology Coordinating Center** | Krischer, Jeffrey P., Ph.D. | H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL |

! **Other Extramural Research Opportunities in Rare Diseases**

ORD is cosponsoring with NIH Institutes the following research activities:

- a Program Announcement with the NHLBI for pilot studies, demonstration projects, and/or exploratory research studies in rare diseases;
- a Request for Applications with the NHGRI for research training grants in genomics and proteomics;
- a Program Announcement with the NINDS to improve treatment outcomes of lysosomal storage disorders and a joint Program Announcement to support grants to provide support for the planning of phase III clinical trials;
- ORD continued its cosponsorship with the National Institute of Diabetes, Digestive, and Kidney Diseases of the Biliary Atresia Research Consortium, which contains nine pediatric liver disease centers. Researchers at the consortium develop and test hypotheses on the cause of biliary atresia and are defining the best means of diagnosis and management of this disease.

*Intramural Research Program*

The Rare Diseases Intramural Research Program is a collaboration between the ORD and the NHGRI at the NIH Clinical Center and supports a number of research activities:

! **Gynecological Aspects of Patients with Rare Diseases**

Intramural researchers established a program to evaluate gynecologic aspects of patients with Hermansky-Pudlak syndrome (HPS), cystinosis, alkaptonuria, and other rare disorders to address the issues that arise when the lives of children with previously fatal rare diseases

are extended into adolescence and adulthood or as adult patients with rare diseases have gynecological complications.

! **Clinical Research Protocols**

Intramural researchers are recruiting patients with Hermansky-Pudlak syndrome (HPS) to enroll in a pirfenidone treatment protocol for the disease's fatal pulmonary fibrosis and in an NIAID sponsored diagnostic protocol for the colitis associated with HPS. HPS is a genetic metabolic disorder that causes albinism; visual impairment; a platelet dysfunction with prolonged bleeding; and progressive symptoms including pulmonary fibrosis, inflammatory bowel disease, and kidney disease.

Intramural research led to the discovery of the gene responsible for Hartnup disorder, a rare inborn error of amino acid reabsorption. Other researchers received approval for clinical protocols, including "Genetic Analysis of Gray Platelet Syndrome (GPS)"; "Investigations of Megakaryocytes from Patients with Abnormal Platelet Vesicles" including Chediak-Higashi syndrome, Griscelli syndrome, and the newly described White platelet syndrome; and "Clinical Investigations into Autosomal Recessive Polycystic Kidney Disease (ARPKD) and Congenital Hepatic Fibrosis" for the possibility of future treatment with a tyrosine kinase inhibitor.

! **Undiagnosed Metabolic Disorders**

The Office of the Clinical Director of NHGRI, with ORD support, has begun to admit patients with rare, undiagnosed disorders. These patients are enrolled in a protocol titled "Diagnosis and Treatment of Patients with Inborn Errors of Metabolism."

! **Diagnostic Tests**

In collaboration with the NHGRI, the ORD supports a program to meet the needs of intramural researchers investigators to establish with CLIA[1]-certified laboratories, molecular diagnostic tests for specific rare diseases. These tests will be made available by the developing laboratory on a fee-for-service, usually insurance-reimbursable basis, to the general public after the needs of the research investigators have been met. The first four genetic tests under development are the human phosphomannose isomerase (MPI) gene and the asparagine-linked glycosylation 6 gene (ALG6) in congenital disorders of glycosylation, the tumor necrosis factor receptor superfamily member 6 (TNFRSF6) gene associate with immune deficiency, and the methylmalonyl coenzyme A mutase (MUT) associated with methylmalonyl-CoA mutase deficiency.

! **Research Training**

[1] Clinical Laboratory Improvement Amendments

In FY 2004, the ORD supported three fellows training in Clinical and Biochemical Genetics, an important field of genetics that brings needed therapy to rare diseases patients. These fellowship opportunities provide for physicians' exposure to rare diseases research early in their careers, reinforcing their interest in and dedication to the field and thereby promoting future research experts in rare diseases. Upon completion of the training program, the research fellows will also be eligible to sit for the examination in biochemical genetics offered by the American College of Medical Genetics (ACMG). Biochemical genetics are important to develop new treatment approaches to inborn errors of metabolism, inherited disorders affecting the production and breakdown of proteins, fats, or carbohydrates.

! **Bench-to-Bedside Intramural Research Awards**

In FY 2004, ORD co-funded with NIH Institutes and Centers 11, 2-year Bench-to-Bedside awards at the NIH Clinical Center. Intramural scientists at the NIH enter into basic science-clinical research collaboration with colleagues in other NIH laboratories with a focus on rare disease. The awards included:

- Therapeutic Application of Intra-Vascular Nitrite for Sickle Cell Disease
- Molecular Profiling of Response to Proteasome Inhibition by Bortezomib (PS341) in a Clinical Trial of Mantle Cell Lymphoma
- A Phase I/II Pilot Study to Evaluate the Treatment of Intraocular Lymphoma with the BL22 Immunotoxin
- A Phase I Treatment Trial of the Circadian Sleep Disturbance in Smith-Magenis Syndrome
- Pre-Clinical Primate Studies of an *In Vivo* Selectable Vector Intended for Use in a Planned Clinical Trial of Gene Therapy for Chronic Granulomatous Disease
- Isolation and Characterization of Circulating Endothelial Cells in Primary Pulmonary Hypertension
- Evaluation of the Humanized MiK-1 Monoclonal Antibody in Patients with HTLV-1 Associated Neurologic Disease
- Molecular Profiling and Drug Discovery for Patients with PTEN Hamartomatous Tumor Syndromes (PHTS)
- Immunotherapy for Myelodysplastic Syndrome
- Intermediate Phenotype and Genetic Mechanisms for Psychosis and Cognitive Disturbance in 22q11.2-hemideletion Syndrome
- Childhood Cancer and Plexiform Neurofibroma Tissue Microarray for Molecular Target Screening and Clinical Drug Development

ORD also continued to support five bench-to-bedside awards in their second year. Those research projects focused on Williams syndrome, hereditary inclusion body myopathy, acute lymphoblastic leukemia, pulmonary sarcoidosis, and leukemogenesis.

**Education, Public Information, and Public Input**

! **Genetic and Rare Diseases Information Center**

The ORD supports with the NHGRI the Genetic and Rare Diseases Information Center (GARD). The information center provides information to patients and their families, health professionals, researchers, and the public. In FY 2004, the information center became more accessible to minority and underserved populations through services in Spanish in addition to English and a more user-friendly Web-based approach. Since its inception in September 2001, the Information Center has responded to more than 10,000 inquiries for more than 3,000 rare diseases.

! **Combined Health Information Database (CHID)**

ORD supported the Medical Genetics and Rare Disorders subfile of the Combined Health Information Database (CHID). The subfile provides information about and available from patient advocacy groups. In 2004 and 2005, ORD is reviewing, updating, and expanding the subfile through a contract with the National Organization for Rare Disorders. At this time, 8,178 indexed documents are in the subfile as well as 1,302 organizations. 4,352 searches were conducted specifically against the subfile in addition to the approximately 1,057,807 searches across CHID as a whole (a total of 11 subfiles.)

**Genetics Education for Healthcare Providers:**

! **National Coalition for Health Professional Education in Genetics (NCHPEG)**

ORD also supported the National Coalition for Health Professional Education in Genetics (NCHPEG). Established in 1996 by the American Medical Association, the American Nurses Association, and the NHGRI, NCHPEG is a national effort to promote health professional education and access to information about advances in human genetics. NCHPEG members are an interdisciplinary group of leaders from approximately 120 diverse consumer and voluntary groups, medical societies, government agencies, private industry, managed care organizations, and genetics professional societies. By promoting frequent and open communication between stakeholders, NCHPEG seeks to capitalize on the collective expertise and experience of members and to reduce duplication of effort. As patients ask more questions about genetic tests and disease risk, more responsibility for the use and interpretation of genetic tests and information will fall to primary care physicians, nurses, physician assistants, advanced practice nurses, and other health professionals who may not be formally trained in genetics. This is of importance to ORD since it is estimated that 80 percent of rare diseases have a genetic basis. Core competency educational materials have been produced in English and in Spanish.

NCHPEG is currently coordinating "Genetic Resources on the Web (GROW)" a source of quality information about human genetics for health professionals and the public. ORD co-founded GROW with other NIH components and Federal agencies. A first training module for genetics for health professionals has been developed for dentists and dental hygienists. The second module is directed toward genetic counselors for a better understanding of genetics and psychiatric disorders. The next module to be completed with the American Academy of Family Physicians will be for physicians in family practice.

**Public Input**

ORD continued to support the annual meetings of the Genetic Alliance and the National Organization for Rare Disorders (NORD). These two umbrella organizations represent collectively more than 600 rare diseases patient advocacy groups. ORD utilized these meetings to conduct focus groups to determine the needs of member organizations and to identify programs ORD should consider implementing. Also, NIH research scientists and ORD staff are active participants in all sessions of the annual meetings and disseminate information about NIH rare diseases research programs.

In FY 2004, ORD continued to support regional workshops to discuss with leaders of patient advocacy groups across the nation all aspects of research and research opportunities in NIH's extramural and intramural research programs. These workshops enable national patient advocacy groups to become partners in research endeavors. To gain public input into ORD program plans, ORD held focus group meetings with leaders of patient advocacy groups at the annual meetings of the NORD and the Genetic Alliance.

**Identifying Future Research Opportunities for Rare Diseases**

ORD collaborates with Institutes, Centers, and Offices at NIH and other Federal agencies to stimulate rare diseases research by cosponsoring scientific conferences where research is lagging or to take advantage of scientific opportunities. The outcomes of these scientific conferences have included the establishment of research priorities, development of collaborative research protocols, criteria for diagnosing and monitoring rare diseases, specific discoveries, publications, and new research endeavors. These scientific conferences have also contributed to the exchange of ideas and information among basic and clinical investigators, patient advocacy groups, NIH staff, and the pharmaceutical industry.

In FY 2004, ORD co-funded 87 national and international scientific conferences. Examples of the scientific conferences in FY 2004 include childhood cancers, bone marrow failure, sickle cell disease, congenital heart disease, dystonias, pediatric stroke, neurofibromatosis, and primary lateral sclerosis. A list of the scientific conferences is provided in the appendix.

**New and Future Initiatives**

ORD has a number of future initiatives and began a number of major new initiatives that will continue and come into fruition in the coming years.

*Coordination of Rare Diseases Research Activities*

! **Trans-NIH Rare Diseases Research Working Group**

The ORD established a Trans-NIH Rare Diseases Research Working Group. Currently the membership encompasses NIH ICs and the Food and Drug Administration's Office of Orphan Products Development. In the future, liaison membership will be expanded to include other Federal agencies and representatives of rare diseases federations. In the coming year, the working group is developing plans for a conference on rare diseases biospecimen collection, storage, and delivery issues that impede research on rare diseases. Other issues under review include expansion of the development of genetic testing in NIH extramural research programs and a conference on amyloidosis.

! **Exploratory and Developmental Research Grants for Investigations in Rare Diseases**

The ORD currently participates with the NHLBI in an announcement for Exploratory and Developmental Research Grants for Investigations in Rare Diseases. The purpose of this announcement defines the scope of exploratory and developmental grant applications to the National Heart, Lung, and Blood Institute (NHLBI) for support of investigators with novel approaches to understanding, treating, and preventing rare diseases in the areas of heart, lung, and blood disease as well as sleep disorders. In FY 2005, ORD will co-fund applications in these rare diseases. Availability of these awards for these individuals is expected to allow investigators with novel ideas to obtain research support without the need for large amounts of preliminary data that often serves as a barrier to entry into the NIH grants system. It is anticipated that these efforts will ultimately result in an increased pipeline of therapeutic approaches to treatment and prevention of rare diseases. Rare diseases are often referred to as "orphan" diseases since there is a general lack of interest among industries to invest resources in diseases that in aggregate comprise too small a population to guarantee return on investment. The ORD would like to expand this program to include other ICs. Partners for each project would be solicited to expand the co-funding throughout the NIH.

! **Diagnostic Testing**

In response to recent concerns about the lack of diagnostic tests for rare diseases, ORD is evaluating this situation. There are more than 6,000 rare diseases, and the majority of the rare diseases are considered genetic, making genetic testing an essential part of the diagnosis and treatment continuum. Currently, genetic testing for rare diseases is only available for a small number of diseases and may only be available through a research laboratory or a laboratory overseas. Developing and marketing tests is not profitable

because of a small number of consumers, and there are no incentives to translate research findings into clinical tests.

To address this need, the ORD initiated in the intramural research program that it sponsors together with the National Human Genome Research Institute a pilot program to bring tests to market by contracting with commercially based and academic center-based, CLIA-certified laboratories.

ORD brought this pilot program to the attention of the Trans-NIH Rare Diseases Research Working Group. The working group was very interested in the outcomes of the pilot program and began discussing means by which such an approach could be applied to extramural programs in the future.

In a parallel move, the ORD collaborated with the Centers for Disease Control and Prevention, Emory University, the American Society for Human Genetics, the American College of Medical Genetics, the Health Resources and Services Administration, and the Genetic Alliance to hold a conference titled "Promoting Quality Laboratory Testing for Rare Diseases: Keys to Ensuring Quality Genetic Testing." Deliberations led to a number of recommendations and planning activities, which will culminate in a follow-up conference to integrate recommendations into actions and projects in 2005. ORD with the ICs will develop a plan to develop the needed infrastructure for the development of genetic tests for research purposes that would meet CLIA standards.

! **Patient Travel to Research and Treatment Sites**

ORD and NHGRI have jointly invited Mercy Medical Airlift to participate in the NIH Clinical Center rare diseases efforts. This not-for-profit organization facilitates transportation free of charge to and from the Clinical Center for patients enrolled in rare disease research protocols and one or two family members. ORD expects expansion of this activity to include the Rare Diseases Clinical Research Network and possibly for travel to research protocols for the U.S. population.

! **Amyloidosis**

Amyloidosis is a group of devastating diseases in which one or more organ systems in the body accumulate abnormal proteins. In primary amyloidosis, the heart, lung, skin, tongue, thyroid gland, and intestinal tract may be involved as well as the liver, spleen, kidney, and the vascular system, especially the heart. Secondary amyloidosis usually affects the spleen, liver, kidney, adrenal glands, lymph nodes, and the vascular system. Hereditary amyloidosis is characterized by peripheral sensory and motor neuropathy, autonomic neuropathy, and cardiovascular and renal involvement. ORD will develop a plan to respond to Congressional concerns and as part of the initiative will cosponsor a conference with NIH ICs.

! **Rare Diseases Biospecimen Repositories**

The issue of availability of high-grade biospecimen and clinical data for research constitutes a barrier to rare diseases research. ORD funded a demonstration project with the National Disease Research Interchange (NDRI) and brought this issue to the attention of the Trans-NIH Rare Diseases Research Working Group. The Genetic Alliance has moved to establish a "biobank" for which it charges patient advocacy groups appropriate fees to participate and to maintain the biospecimens that are DNA. However, many rare diseases groups do not have the funds for such an endeavor, and many more rare diseases do not have an advocacy group. A number of organizations have expressed interest in accommodating rare diseases research, but a comprehensive examination of the issues is needed. The Trans-NIH Rare Diseases Research Working Group has formed a planning committee to determine the issues that need to be explored and to identify the significant players of biospecimen collection, storage, and dissemination. The planning committee will provide recommendations to the NIH for a conference that will lead to a clear understanding of the needs and a plan for implementation.

**Identifying Future Research Opportunities for Rare Diseases**

In FY 2005, ORD will cosponsor approximately 100 scientific conferences. At this time, 58 scientific conferences have been approved (see Table 3 in the appendix for a list of topics), with another two application cycles throughout FY 2005.

**Genetic and Rare Diseases Education**

! **Rare Diseases Information**

The ORD Web site provides information about biomedical research, scientific conferences, and rare diseases and is a portal to information on major topics of interest in the rare diseases community. The ORD will participate in a Web site user satisfaction survey to further improve the outreach to and delivery of information to patients with rare diseases.

The Genetic and Rare Information Center will provide additional services in Spanish and will, together with ORD and NHGRI, continue outreach efforts to underserved populations.

! **Education of Leaders of Patient Advocacy Groups**

In January 2005, ORD will conduct a regional workshop in Philadelphia for 55 leaders of national patient support organizations. In the past, ORD conducted regional workshops in New York and in San Francisco for more than 85 leaders of national patient advocacy groups to assist the support groups in being an active participant in the rare diseases research enterprise by getting a better understanding of the structure and function of the NIH and the Food and Drug Administration (FDA).

! **A Database of National and International Patient Advocacy Organizations**

ORD supports the Medical Genetics and Rare Disorders subfile of the Combined Health Information Database (CHID). In 2005 and 2006, ORD is reviewing, updating, and

expanding the subfile to include English and Spanish speaking international patient advocacy organizations and their publications, through a contract with the National Organization for Rare Disorders. The database will expand to include many more international organizations with an emphasis on rare diseases.

**Outreach to the Latino Communities**

The National Human Genome Research Institute, the ORD, and The National Council of La Raza's Institute for Hispanic Health, will partner in a pilot education project to demonstrate efficient ways to reach Latino communities with information about genetic and rare diseases.

**Appendix:**

## Table 2: ORD Cosponsored Scientific Conferences in FY 2004

| Primary Cosponsor | Title |
|---|---|
| **NIA** | • Cockayne Syndrome and Related Disorders of DNA Repair and Transcription: From Bench to Bedside and Back |
| **NIAAA** | • Mechanisms of Alcohol-associated Cancer Symposium |
| **NIAID** | • Transplantation Immunology<br>• International Conference on Burkholderia Pathogenesis: Approaches and Opportunities for Research on Glanders and Melioidosis |
| **NIAMS** | • Biology of Calpains in Health and Disease<br>• Intermediate Filaments<br>• Mild Osteogenesis Imperfecta: Toward Better Understanding and Treatment<br>• New Directions in Biology and Disease of Skeletal Muscle<br>• Pathogenic Mechanisms of Fibrosis: Search for Common Ground<br>• Pseudoxanthoma Elasticum Research Meeting 2004: Building on the Basics |
| **NCI** | • 19th International Pigment Cell Conference: A Focus on Human Pigmentary Disorders<br>• Thrombospondins and Other Modulatory Adhesion Molecules in Tissue Organization<br>• ATP Binding Cassette Gene<br>• Biliary Tract Cancer<br>• Cancer Survivorship: Genetic Susceptibility and Second Primary Cancers<br>• Childhood Cancer: Improving Care After Treatment<br>• Collaborative Approaches to Discovering Genes in African Americans and Hispanics Utilizing Mapping by Admixture Linkage Disequilibrium<br>• First NIH Peritoneal Mesothelioma Meeting<br>• Global Increases in Esophageal Adenocarcinoma: Current Epidemiologic Research and Future Directions |

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| | • Lymphangiogenesis and Cancer Workshop<br>• NIH Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-host Disease<br>• RNA Interference: Target Validation and Potential Therapeutic Applications for Childhood Cancers<br>• Sarcoma and Mesenchymal Stem Cell Biology |
| **NICHD** | • Malformation/Morphogenesis<br>• Children with Special Needs: Planning a Research Future<br>• Collaborative Genetic Disease Research: Needs and Opportunities<br>• Cornelia de Lange Syndrome<br>• Fetal Therapy: Needs Assessment and Future Directions<br>• Molecular Aspects of Infection and Inflammation—Common Pathways, Rare Pediatric Diseases<br>• Pineal Cell Biology<br>• Reproduction and the Fragile X Premutation<br>• Signaling in Vertebrate Organogenesis<br>• The World Congress on Chromosome Abnormalities |
| **NIH Clinical Center** | • Functional Genomics of Critical Illness and Injury |
| **NIDCD** | • Universal Reporting Parameters for the Speech of Individuals with Cleft Palate |
| **NIDDK** | • Protein Misfolding and Misprocessing in Disease<br>• Multiple Endocrine Neoplasias<br>• Workshop on Bioiron, Thalassemia, Sickle Cell Disease, and Hemochromatosis |
| **NIDCR** | • Advancing Diagnostic Approaches for TMJ Diseases and Disorders |
| **NIEHS** | • Assessing Human Germ Cell Mutagenesis in the Post-genome Era<br>• Environmental Neuroscience<br>• Mold-related Health Effects: Clinical, Remediation Worker Protection, and Biomedical Research Issues |

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| **NEI** | • Central Nervous System Lymphoma<br>• The First International Symposium on Translational Clinical Research for Inherited and Orphan Retinal Diseases |
| **NHLBI** | • International Pulmonary Hypertension<br>• A Consultative Network for Sickle Cell Disease<br>• Bone Marrow Failure<br>• Frontiers of Knowledge in Sleep and Sleep Disorders: Opportunities for Improving Health and Quality of Life<br>• Inflammation and Immunity in Dilated Cardiomyopathy<br>• Molecular Mechanisms in Lymphatic Function and Disease<br>• Needs and Opportunities to Study Hypersensitivity Pneumonitis<br>• Pediatric Interstitial Lung Disease International Research Conference<br>• Second Annual International Pulmonary Alveolar Proteinosis Research Conference<br>• The Lymphangioleiomyomatosis Foundation International Research Conference<br>• Research in Adult Congenital Heart Disease |
| **NHGRI** | • Engaging Latino Communities in the Future of Genomics Science<br>• Gene Therapy for Wiskott-Aldrich Syndrome: Current Status and Plans for the Future<br>• New Direction for Sickle Cell Therapy in the Genome Era<br>• Holoprosencephaly (HPE): Midline and Laterality<br>• First NIH-ORD Conference on Cystinosis: Past, Present, and Future |
| **NIMH** | • First International Symposium on N-Acetylaspartate (Canavan Disease)<br>• Pragmatic Considerations of Culture and Prevention of Suicide |

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|---|---|
| **NINDS** | • Rehabilitative Approaches to Dystonia<br>• WORLD Lysosomal Diseases Clinical Research Network<br>• Brain Uptake and Utilization of Fatty Acids, Lipids, and Lipoproteins: Applications to Neurological Disorders<br>• Calcium and Cell Function<br>• Clinical Trials in Spinal Muscular Atrophy<br>• Developing Therapies for the Neurofibromatoses<br>• Neurofibromatosis Research Program<br>• Drug Screening for Ataxia-Telangiectasia<br>• First International Conference on Ideomotor Apraxia<br>• Pathogenesis and Treatment of Periodic Paralyses<br>• Scientific and Clinical Symposium on Tourette Syndrome<br>• Frontotemporal Dementia and Pick's Disease<br>• Glutamic Acid Decarboxylase Autoimmunity in Batten Disease<br>• Rare Neuroimmunologic Disorder<br>• The Glycoproteinoses: An International Workshop on Advances in Pathogenesis and Therapy<br>• Developmental Brain Injury<br>• International Trial Network on Duchenne Muscular Dystrophy<br>• Primary Lateral Sclerosis<br>• Toward the Development of Pediatric Stroke Trials |
| **NINR** | • Developing the Capacity for End of Life and Palliative Care Research<br>• Increasing Opportunities in Biobehavioral Research Utilizing Allergic Bronchopulmonary Aspergillosis as a Framework<br>• Promoting Research on Focal Cognitive Deficits in Non-dementing Disorders |
| **CDC** | • Promoting Quality Laboratory Testing for Rare Diseases: Key to Insuring Quality Genetic Testing |

**Table 3: ORD Cosponsored Scientific Conferences so far in FY 2005**

| Primary Cosponsor | Title |
|---|---|
| **NIA** | • Human Mitochondrial Diseases |
| **NIAAA** | • Mechanisms of Alcohol-induced Hepatic Fibrosis |
| **NIAID** | • Pulmonary Nontuberculous Mycobacterial Infections: Phenotype and Genotype of an Autosomal Dominant Disorder Primarily Affecting Older Women<br>• The Molecular Biology of Spirochetes<br>• Gordon Conference on the Biology of Spirochetes<br>• Treatment of Neurocysticercosis<br>• Eosinophil-associated Disease: Approaches to Treatment<br>• Collaborative Immune Studies in Leprosy<br>• Hydatidosis–New Approaches to Vaccines and Prevention<br>• Human African Trypanosomiasis: Strategic Research Direction |
| **NIAMS** | • Burden of Muscle Disease<br>• The 4th International Congress on Systemic Autoinflammatory Diseases |
| **NCI** | • NCI-MMHCC Working Group on Brain Tumors<br>• Kidney Cancer–Current Perspectives and Future Directions<br>• Translational Genomics of Neuroblastoma<br>• NIH Development Project on Criteria for Clinical Trials in Chronic Graft-versus-host Disease–Final Open Conference<br>• Biology and Therapy for Malignant Salivary Gland Tumors<br>• Cancer Risk in Relatives in Ataxia-Telangiectasia Patients<br>• Liver Cancer in the United States<br>• Bloom Syndrome: Molecular Basis of Genomic Instability<br>• Poverty and Cancer: AIDS-Related Malignancy |

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| **NICHD** | • Mechanisms of Follicular Dysfunction in Ovarian Insufficiency and Premature Ovarian Failure<br>• First International Symposium on Pheochromocytoma<br>• Artificial Reproductive Technology and Adverse Pregnancy Outcomes<br>• 9th International Conference on Osteogenesis Imperfecta<br>• Oxygen in Babies<br>• Collaborative Genetic Disease Research: Needs and Opportunities<br>• Near-term Pregnancy and Near-term Newborn Infant: Optimizing Care and Long-term Outcomes |
| **NIH Clinical Center** | • Role of Nitrite in Physiology, Pathophysiology, and Therapeutics |
| **NIDCR** | • Sjögren's: Transition from Autoimmunity to Lymphoma |
| **NIDDK** | • Familial Amyloidotic Polyneuropathy and Other Transthyretin Related Disorders and the Liver Transplantation in Familial Amyloidotic Polyneuropathy<br>• Primary Sclerosing Cholangitis: Research Workshop<br>• Inherited Metabolism Disorders |
| **NIEHS** | • Gene/Environment Interactions in Rare Diseases that Include Common Birth Defects<br>• Ion Channel Regulation<br>• Conference on Environmental Mutagens |
| **NIGMS** | • Physiological Genomics of Critical Illness |
| **NHLBI** | • Resources for Late-stage Drug Development for Hemoglobin Disorders<br>• Refinement of Hemoglobin Gene Transfer Vectors and Approaches for Clinical Application to Sickle Cell Disease and Cooley's Anemia<br>• Hemoglobinopathy Working Group<br>• Meeting of the National Sickle Cell Disease Program<br>• Cooley's Anemia Symposium<br>• International Kawasaki Disease Symposium<br>• Cardiovascular Development Conference<br>• Working Group on Cardiomyopathies of Rare Diseases |

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| **NHGRI** | • Hermansky-Pudlak Syndrome<br>• Autosomal Recessive Polycystic Kidney Disease<br>• Smith-Magenis Syndrome Research Roundtable Symposium |
| **NIMH** | • Indigenous Suicide in the Americas |
| **NINDS** | • Tuberous Sclerosis Complex<br>• Neurodegeneration with Brain Iron Accumulation<br>• Neuronal Ceroid Lipofuscinosis<br>• CAG Triplet Repeat Disorders<br>• Research Planning Workshop on Spasmodic Dysphonia<br>• Hydrocephalus: Myths, New Facts, Clear Directions<br>• Vascular Cognitive Impairment: Harmonization Criteria |
| **NINR** | • Research Issues in a Multicultural Society HIV/AIDS as a Case Study |
| **ODS** | • Dietary Supplements, Coagulation, and Antithrombotic Therapies |

**Index**

Acronyms

| | |
|---|---|
| a-1 | alpha-one |
| AA | aplastic anemia |
| AAMDSIF | Aplastic Anemia & MDS International Foundation |
| AAT | a1-antitrypsin, |
| AATD | a1-antitrypsin deficiency |
| ABPA | allergic bronchopulmonary aspergillosis |
| ACCESS | A Case Control Etiologic Study of Sarcoidosis (NHLBI) |
| ACMG | American College of Medical Genetics |
| ADCC | Autoimmune Diseases Coordinating Committee |
| ADHD | attention deficit hyperactivity disorder |
| AFSP | American Foundation for Suicide Prevention |
| AGS | Alagille syndrome |
| ALD | adrenoleukodystrophy (NLM) |
| ALL | (childhood) acute lymphoblastic leukemia |
| ALPS | autoimmune lymphoproliferative syndrome |
| ALS | amyotrophic lateral sclerosis |
| A/M | anophthalmia/microphthalmia |
| APS | antiphospholipid syndrome |
| ARDS | acute respiratory distress syndrome |
| ARND | alcohol-related neurodevelopmental disorder |
| ARVD | arrhythmogenic right ventricular dysplasia |
| AS | Angelman syndrome |
| ASCUS | atypical squamous cells of undetermined significance |
| ASF | Angelman Syndrome Foundation |
| ASPS | advanced sleep phase syndrome |
| AT | ataxia telangiectasia |
| BAA | broad agency announcement (NHLBI) |
| BBS | Bardet-Biedl syndrome |
| BDNF | brain-derived neurotrophic factor |
| BE | Barrett’s esophagus |
| BH4 | tetrahydrobiopterin |
| BIBIN | bilirubin-induced brain injury in the newborn |

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|---|---|
| BLM | human gene encoding Bloom syndrome |
| BN | bulimia nervosa |
| BPD | bronchopulmonary dysplasia |
| BS | Bloom syndrome |
| BSE | bovine spongiform encephalopathy |
| CAG (triplet repeat) | nucleotides (CAG) consecutively repeated within a region of DNA |
| CAM | complementary and alternative medicine |
| CASG | Collaborative Antiviral Study Group |
| CASPAR | computerized affected sibling pair analyzer and reporter |
| CBV | coxsackie virus B |
| CC | Warren Grant Magnuson Clinical Center, NIH |
| CCHS | congenital central hypoventilation syndrome |
| CDC | Centers for Disease Control and Prevention |
| CDG | congenital disorders of glycosylation |
| CDH | congenital diaphragmatic hernia |
| CF | cystic fibrosis |
| CFS | chronic fatigue syndrome |
| CFTR | cystic fibrosis (CF) transmembrane conductance regulator |
| CGD | chronic granulomatous disease |
| CHD | coronary heart disease |
| CHID | Combined Health Information Database |
| CHOP | Children's Hospital of Philadelphia |
| CIN | cervical intraepithelial neoplasia |
| CJD | Creutzfeldt-Jakob disease |
| CLL | chronic lymphocytic leukemia |
| CL/P | cleft lip and cleft palate |
| CMV | congenital cytomegalovirus |
| CNS | central nervous system |
| COPD | chronic obstructive pulmonary disease |
| CRADA | cooperative research and development agreement |
| CRC | clinical research center |
| CRD | cannabis-related disorder |
| CRF | corticotropin-releasing factor |
| CS | Cockayne syndrome |
| CVB3 | Cocksackie virus B3 |
| CWD | chronic wasting disease |
| DCIPS | Developing Centers on Interventions for the Prevention of Suicide (NIMH) |
| DDG | Drug Development Group (NIH) |
| DeNOVO | delivery of NO for vaso-occlusion (clinical trial title) |
| DHHS | Department of Health and Human Services |

| | |
|---|---|
| DMAC | disseminated infection with mycobacterium avium complex |
| DNA | deoxyribonucleic acid |
| DOE | Department of Energy |
| DSRCT | desmoplastic small round-cell tumor |
| DTCC | Data and Technology Coordinating Center |
| EB | epidermolysis bullosa, severe blistering skin diseases |
| EBV | Epstein-Barr virus |
| ECMO | extracorporeal membrane oxygenation |
| EDS | Ehlers-Danlos syndrome |
| ES | Ewing's sarcoma |
| FA | Fanconi anemia |
| FAS | fetal alcohol syndrome |
| FASD | fetal alcohol spectrum disorders |
| FBN1 | fibrillin 1 |
| FDA | Food and Drug Administration |
| FENIB | familial encephalopathy with neuronal inclusion bodies |
| FGFR3 | fibroblast growth factor receptor 3 |
| FH | familial hypercholesterolemia |
| FHBL | familial hypobetalipoproteinemia |
| FMR1 | fragile X mental retardation gene |
| FRDA | Friedreich ataxia |
| FRX | fragile X syndrome |
| FSHD | facio-scapulo-humeral dystrophy |
| GCPS | Greig cephalopolysyndactyly syndrome |
| GCRC | General Clinical Research Center (NCRR) |
| GHR | Genetics Home Reference (NLM) |
| GLP | good laboratory practice |
| GMP | good manufacturing practice |
| GPS | Gray platelet syndrome |
| HAART | highly active anti-retroviral therapy |
| HbF | fetal hemoglobin |
| HD | Huntington disease |
| HDL | high-density lipoprotein |
| HEV | hepatitis E virus |
| HGP | human genome project |
| HGPS | Hutchinson-Gilford progeria syndrome |
| Hh | hedgehog (signaling pathway) |
| HHT | hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) |
| HHV-8 | human herpesvirus 8 |
| HIBM | hereditary inclusion body myopathy |
| hIPF | hereditary idiopathic pulmonary fibrosis |
| HIV/AIDS | human immunodeficiency virus/acquired immune deficiency syndrome |

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|---|---|
| HPP | health partnership program (NIAMS) |
| HPT-JT | hyperparathyroidism-jaw tumor (syndrome) |
| HPV | human papillomavirus |
| HRSA | Health Resources and Services Administration |
| HTLV | human T cell leukemia virus |
| IBMFS&C | idiopathic bone marrow failure states and cytopenias |
| IBS | irritable bowel syndrome |
| ICBG | International Cooperative Biodiversity Groups (NHLBI) |
| ICs | (NIH) institutes and centers |
| IDDM | insulin-dependent diabetes mellitus |
| IFN | interferon |
| IGFs | insulin-like growth factors |
| IL | interleukin |
| IND | investigational new drug |
| IPF | idiopathic pulmonary fibrosis |
| IRSA | International Rett Syndrome Association |
| ISIS | Imaging Science and Information Systems Center (NLM) |
| JDRF | Juvenile Diabetes Research Foundation International |
| JRA | juvenile rheumatoid arthritis |
| KTWS | Klippel-Trenaunay-Weber syndrome |
| LAM | lymphangioleiomyomatosis |
| LDL | low-density lipoprotein |
| LMNA | lamin A (gene) |
| LQTS | long QT syndrome |
| LVAD | left ventricular assist device |
| mAB | monoclonal antibodies |
| MADGC | Multiple Autoimmune Diseases Genetics Consortium |
| MALD | mapping by admixture linkage disequilibrium |
| MATT | Methamphetamine Addiction Treatment Think Tank (NIDA) |
| MCA/MR | multiple congenital anomaly/mental retardation |
| MD-CARE | P.L. 107-84, Muscular Dystrophy Community Assistance, Research, and Education Amendments of 2001 |
| MDCC | Muscular Dystrophy Coordinating Committee |
| MDS | myelodysplastic syndrome |
| MDA | Muscular Dystrophy Association |
| MDD | Medications Development Division (NIDA) |
| MDMA | Methylene-dioxy-meth-amphetamine (ecstasy) |
| MEN1 | multiple endocrine neoplasia type 1 |
| MHC | major histocompatibility complex |
| MKS | McKusick-Kaufman syndrome |
| MMP | matrix metalloproteinase |
| MOU | Memorandum of Understanding |

| | |
|---|---|
| MPD | myeloproliferative disease |
| MR4 | Malaria Research and Reference Reagent Resource (Center) |
| MS | multiple sclerosis |
| MSC | mesenchymal stem cell |
| MSH | Multicenter Study of Hydroxyurea |
| MTA | material transfer agreement |
| NBLs | National Biocontainment Laboratories |
| NBN | National Biospecimen Network |
| NBTT | New Approaches to Brain Tumor Therapy Consortium (NCI) |
| NCBI | National Center for Biotechnology Information |
| NCCAM | National Center for Complementary and Alternative Medicine |
| NCI | National Cancer Institute |
| NCL | neuronal ceroid lipofuscinosis (Batten disease) |
| NCMHD | National Center on Minority Health and Health Disparities |
| NCRR | National Center for Research Resources |
| NCS | National Children's Study |
| NDA | new drug application |
| ND-BD | non-dementing brain disorders |
| NEI | National Eye Institute |
| NF1 | neurofibromatosis type 1 |
| NF-kappaB | nuclear factor kappaB |
| NGI | next generation Internet |
| NHGRI | National Human Genome Research Institute |
| NHL | non-Hodgkin lymphoma |
| NHLBI | National Heart, Lung, and Blood Institute |
| NIA | National Institute on Aging |
| NIAAA | National Institute on Alcohol Abuse and Alcoholism |
| NIAID | National Institute of Allergy and Infectious Disease |
| NIAMS | National Institute of Arthritis and Musculoskeletal and Skin Diseases |
| NICHD | National Institute of Child Health and Human Development |
| NIDA | National Institute on Drug Abuse |
| NIDCD | National Institute of Deafness and Other Communication Disorders |
| NIDCR | National Institute of Dental and Craniofacial Research |
| NIDDK | National Institute of Diabetes and Digestive and Kidney Diseases |
| NIEHS | National Institute of Environmental Health Sciences |
| NIH | National Institutes of Health |
| NIMH | National Institute of Mental Health |
| NINDS | National Institute of Neurological Disorders and Stroke |
| NINR | National Institute of Nursing Research |
| NLM | National Library of Medicine |
| NNFF | National Neurofibromatosis Foundation |
| NO | nitric oxide |

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| NOMID | neonatal onset multisystem inflammatory disease |
| NPA | Niemann-Pick type A disease |
| NPB | Niemann-Pick type B disease |
| NPC | Niemann-Pick type C disease |
| NPD | Niemann-Pick type D disease |
| OA | osteoarthritis |
| OCRL | oculo-cerebro-renal syndrome (Lowe syndrome, LS) |
| ODS | Office of Dietary Supplements (Office of the Director, NIH) |
| OI | osteogenesis imperfecta |
| ORD | Office of Rare Diseases (Office of the Director, NIH) |
| ORWH | Office of Research on Women's Health (Office of the Director, NIH) |
| OTP | opiate treatment program (NIDA) |
| PA | program announcement |
| PACT-G | Pediatric AIDS Clinical Trial Group |
| PAP | pulmonary alveolar proteinosis |
| PCD | primary ciliary dyskinesia |
| PCP | phencyclidine |
| PCP | pneumocystis carinii pneumonia |
| PEGT | Programs of Excellence in Gene Therapy (NHLBI) |
| PGA | Programs for Genomic Applications (NHLBI) |
| PHS | Pallister-Hall syndrome |
| PKC | protein kinase C |
| PKU | phenylketonuria |
| PML | progressive multifocal leuceoencephalopathy |
| POF | premature ovarian failure |
| PPH | primary pulmonary hypertension |
| PPHN | persistent pulmonary hypertension of the newborn |
| PTLD | post-transplant lymphoproliferative disease |
| PTS | post-traumatic stress syndrome |
| PWS | Prader-Willi syndrome |
| PXE | pseudoxanthoma elasticum |
| RA | rheumatoid arthritis |
| RAID | Rapid Access to Intervention Development Program (NCI) |
| RBLs | regional biocontainment laboratories |
| RDCRC | Rare Disease Clinical Research Consortium |
| RDCRN | Rare Diseases Clinical Research Center Network |
| REM | rapid eye movement (characteristic of deep sleep) |
| RFA | request for applications |
| RFP | request for proposals |
| RLGS | restriction landmark genome scanning |
| RNA | ribonucleic acid |
| RS | Rett syndrome |

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| RTH | resistance to thyroid hormone |
| RTOG | Radiation Therapy Oncology Group |
| RTS | Rothmund-Thompson syndrome |
| SADDAN | severe achondroplasia with developmental delay and acanthosis nigricans |
| SAMHSA | Substance Abuse and Mental Health Services Administration |
| SAGA | Sarcoidosis Genetic Analysis Consortium |
| SARS | severe acute respiratory syndrome |
| SBIR | small business innovative research |
| SCD | sickle cell disease |
| SCD | sudden cardiac death |
| SCID | severe combined immunodeficiency disorder |
| SCOR | specialized center of research |
| SGBS | Simpson Golabi Behmel syndrome |
| SIDS | sudden infant death syndrome |
| SLE | systemic lupus erythematosus |
| SLOS | Smith-Lemli-Opitz syndrome |
| SMA | spinal muscular atrophy |
| SPORE | Specialized Program of Research Excellence |
| SUD | substance use disorder |
| SS | sickle cell |
| SS | Sjögren syndrome |
| SVAS | supravalvular aortic stenosis |
| TB | tuberculosis |
| TCR | transcription-coupled repair |
| TIGR | The Institute for Genomic Research |
| TMAU | trimethylaminuria |
| TMD | temporo-mandibular disorders |
| TMJ | temporomandibular joint |
| TSC | tuberous sclerosis complex |
| TSEs | transmissible spongiform encephalopathies |
| TTP | thrombotic thrombocytopenic purpura |
| UCD | urea cycle disorder |
| UIP | usual interstitial pneumonitis |
| UPD | uniparental disomy |
| UV | ultraviolet |
| VA | (Department of )Veterans Affairs |
| vCFD | variant Creutzfeldt-Jakob disease |
| VCFS | velo-cardio-facial syndrome |
| VCRN | Vasculitis Clinical Research Network |
| VEG5Q | vascular endothelial gene on chromosome 5q |
| VEGF | vascular endothelial growth factor |
| VLBW | very low birth weight |

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| VLDL | very low-density lipoprotein |
| VTUs | vaccine treatment and evaluation units |
| VWD | von Willebrand disease |
| VWF | von Willebrand factor |
| WAS | Wiscott-Aldrich syndrome |
| WNV | West Nile virus |
| WRN | defective gene for Werner syndrome |
| WS | Waardenburg Syndrome (NIDCD) |
| WS | Werner syndrome (NIA) |
| WS1 | Wilm’s tumor suppressor |
| XPD | a human DNA repair protein |
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